ANNALS OF SAUDI MEDICINE

VOLUME 30 ISSUE 1 JANUARY-FEBRUARY 2010 MUHARRAM-RABIA AL-AWAL 1431

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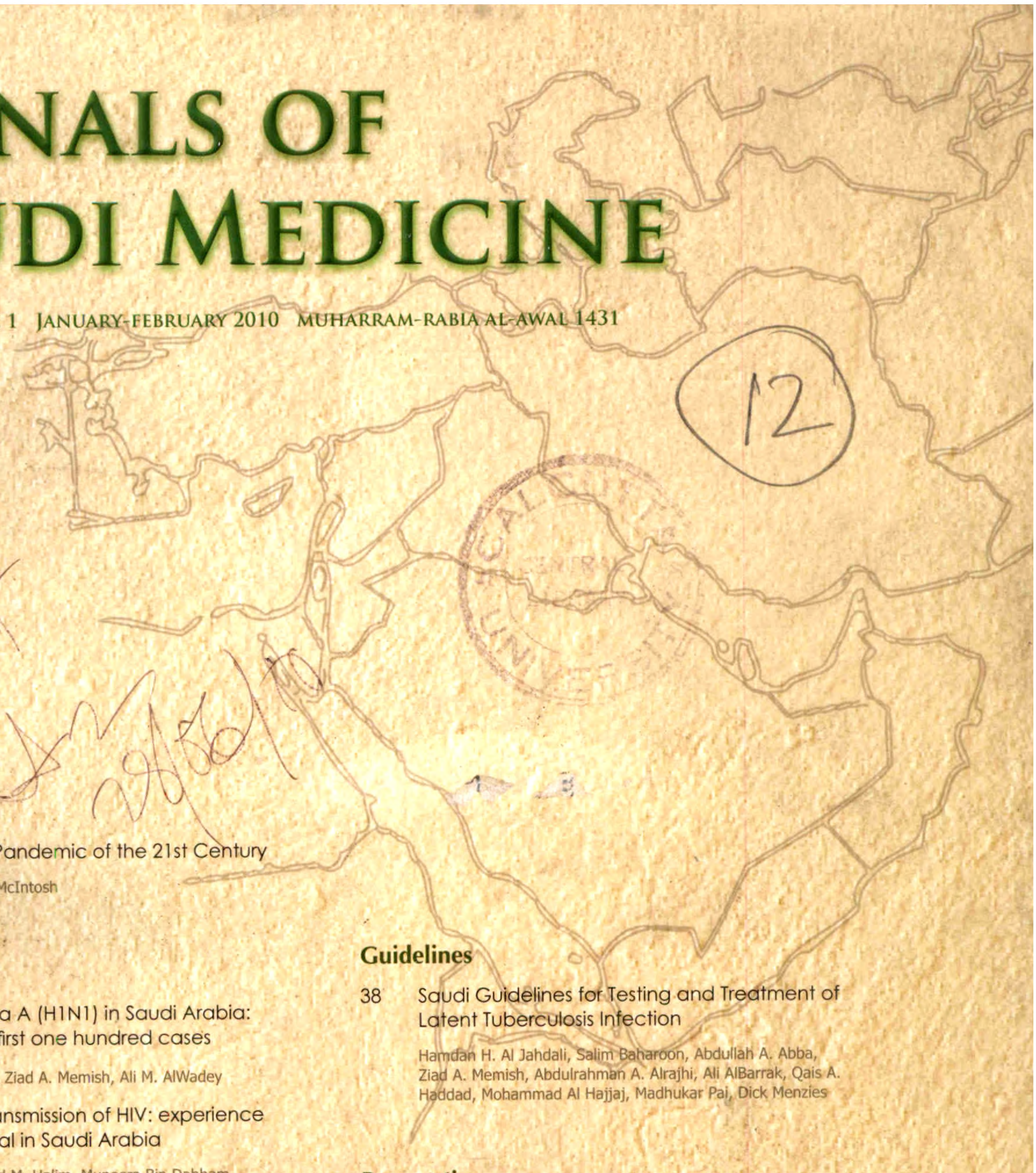

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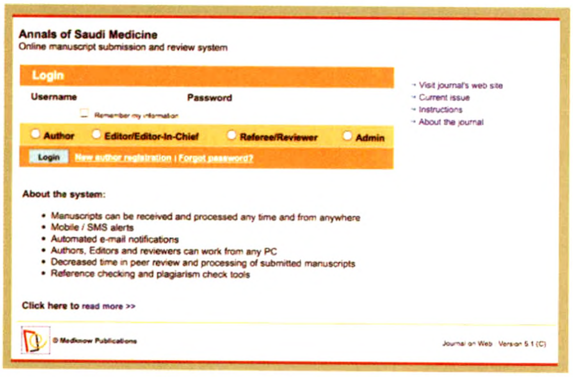

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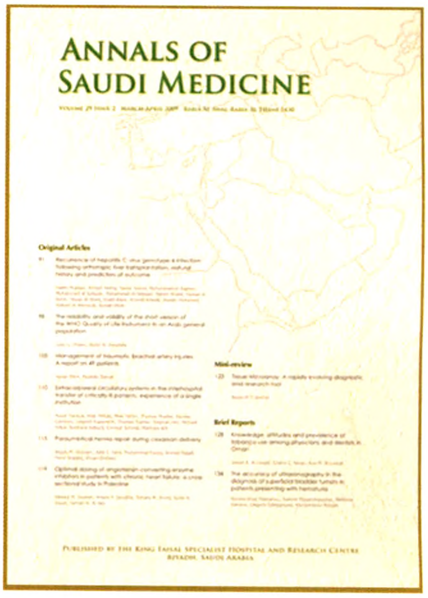

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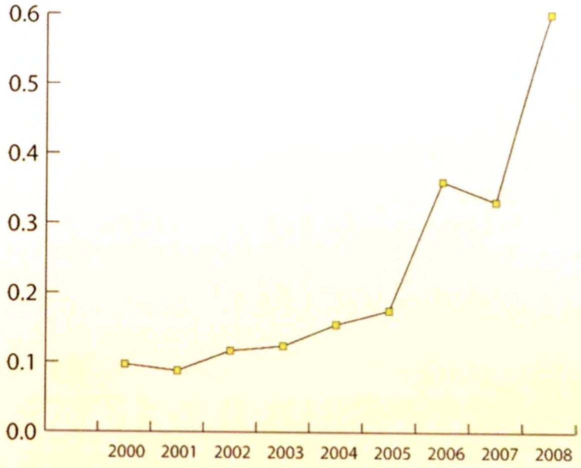

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ACADEMIC CALENDAR

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JANUARY (1)

3rd KFSH&RC Residents' Research Day
20 January 2010 / 05 Safar 1431
Contact No.: 4647272 Ext 31730 (Research Center)

MARCH (6)

2nd International Conference on "Radiation Medicine: Innovative Approaches & Interprofessional Practice"
01-04 March 2010 / 15-18 Rabi Al Awal 1431
Contact No.: 4427879 (Biomedical Physics, Research Center)

1st Middle East Symposium on Diseases of the Esophagus
09-10 March 2010 / 23-24 Rabi Al Awal 1431
Contact No.: 4647272 Ext. 32958 (Surgery)

22nd Saudi Urological Conference
15-18 March 2010 / 29 Rabi I – 02 Rabi II 1431
Contact No. 4647272 Ext. 31830 or 4424302 (Urology)

1st International Conference in Pediatric Emergencies, Injury & Disability Prevention
23-25 March 2010 / 07-09 Rabi Al Thani 1431
Contact No.: 4424425 (Emergency Medicine)

Annual Saudi Colorectal Surgery Forum
27-31 March 2010 / 11-15 Rabi Al Thani 1431
Contact No.: 4427754 (Surgery)

3rd Mini AABB Symposium
30 March – 01 April 2010 / 14-16 Rabi Al Thani 1431
Contact No.: 4424224 (Pathology & Laboratory Medicine)

APRIL (3)

1st Annual Pediatric Nephrology Meeting
06-07 April 2010 / 21-22 Rabi Al Thani 1431
Contact No.: 4427798 (Pediatrics)

Medication Safety: A Mission for All Symposium
13-14 April 2010 / 28-29 Rabi Al Thani 1431
Contact No.: 4427601(Pharmacy Services Division)

International Symposium on New Frontiers in Breast Cancer
27-29 April 2010 / 13-15 Jumada Al Awal 1431
Contact No. 4647272 Ext. 31830 or 4423940 (Oncology Center)

MAY (7)

Conference on Diagnostic & Prognostic Testing in Clinical Biochemistry – A New Age of Disease Biomarkers
04-05 May 2010 / 20-21 Jumada Al Awal 1431
Contact No.: 4427224 (Pathology & Laboratory Medicine)

Oncology Centre Academic & Research Day
05 May 2010 / 21 Jumada Al Awal 1431
Contact No.: 4647272 Ext 38005 Oncology Center

Preventative Genetics: New Initiatives Workshop
11-12 May 2010 / 27-28 Jumada Al Awal 1431
Contact No.: 4424988 (Medical Genetics)

12th Clinical Research Professionals' Course
15-26 May 2010 / 01-12 Jumada Al Thani 1431
Contact No.: 4424987
(Centre for Clinical Studies and Empirical Ethics)

Special Care Dentistry Symposium
18-19 May 2010 / 04-05 Jumada Al Thani 1431
(Workshop on 20 May 2010 / 06 Jumada Al Thani 1431)
Contact No. 4647272 Ext. 31830 or 4427652 (Dentistry)

Ultrasound Guided Regional Anesthesia & Vascular Access Workshop
26-27 May 2010 / 11-12 Jumada Al Thani 1431
Contact No.: 4647272 Ext. 32680 (Anesthesiology)

8th Annual Academic Day
26 May 2010 / 12 Jumada Al Thani 1431
Contact No.: 4423934 (Pediatric Hematology / Oncology)

JUNE (2)

3rd Pediatric Infectious Diseases Seminar
01-02 June 2010 / 18-19 Jumada Al Thani 1431
Contact No.: 4427761 (Pediatrics)

5th LC & LCMS: Concept & Hands-On Training
05-09 June 2010 / 22-26 Jumada Al Thani 1431
Contact No.: 4424987
(Centre for Clinical Studies and Empirical Ethics)

SEPTEMBER (1)

International Symposium on Adult Spine Tumor
28-29 September 2010 / 19-20 Shawwal 1431
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OCTOBER (8)

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Contact No.: 4427763 (Pediatrics)

Internal Medicine Saudi Board Review Course
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12-13 October 2010 / 04-05 Dhu Al Qada 1431
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13th Clinical Research Professionals' Course
16-27 October 2010 / 08-19 Dhu Al Qada 1431
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Adult Congenital Heart Disease Awareness Day
18 October 2010 / 10 Dhu Al Qada 1431
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Congenital Heart Disease Symposium
19-20 October 2010 / 11-12 Dhu Al Qada 1431
Contact No. 4647272 Ext. 31830 or 32452
(King Faisal Heart Institute)

2nd International Pain Management Symposium
26-28 October 2010 / 18-20 Dhu Al Qada 1431
Contact No.: 4647272 Ext. 32680 (Anesthesiology)

10th Annual Surgical Research & Residents Day
28 October 2010 / 20 Dhu Al Qada 1431
Contact No.: 4427753 (Surgery)

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A New Vision in Medical Nutrition Symposium
30 Nov – 01 Dec 2010 / 24-25 Dhu Al Hijja1431
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01 December 2010 / 25 Dhu Al Hijja 1431
Contact No.: 4427494 (Medicine)

Treatment Modalities for Genetic Diseases: From Diet to Gene Therapy Workshop
07-08 December 2010 / 01-02 Muharram 1432
Contact No.: 4424988 (Medical Genetics)

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25-29 December 2010 / 19-23 Muharram 1432
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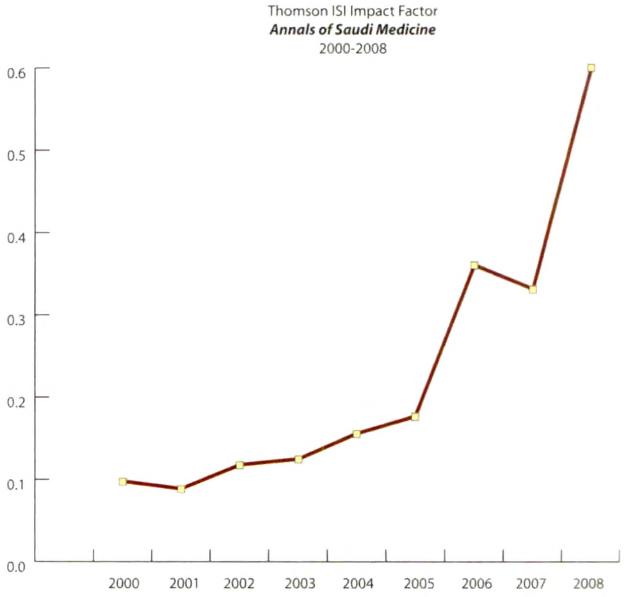
FROM THE EDITOR-IN-CHIEF

This issue of the *Annals* is the first of Volume 30, marking three decades of publication in service to the local and regional medical community. Initially intended as a vehicle for publishing the medical experiences of physicians at King Faisal Specialist Hospital, the journal has grown to become one of the most important general medical journals, not only in the Middle East, but throughout the Eastern Mediterranean and Asia, with manuscripts submitted from all over the world. Our new entry on Wikipedia (*http://en.wikipedia.org/wiki/Annals_of_Saudi_Medicine*) tells more about the founding and history of the *Annals*.

One of the most significant achievements was the remarkable increase in the Impact Factor, the most widely used measure of progress of a publication. For 2008, it nearly doubled again from the previous year. The *Annals* now has by far the highest Impact Factor in the region. Since we have made no attempt to artificially increase the Impact Factor, we believe this increase is due solely to continuing improvement in the importance of articles we publish, which is a reflection of the efforts of all involved in the publication of the journal. Of the nearly 1000 manuscripts submitted to the *Annals* each year, about 13 to 15 percent pass the rigorous peer review process and are accepted for publication.

Another important development in the past year is the conversion to use of a combination manuscript management and electronic publishing system using the services of Medknow Publications. We believe use of this system will help to streamline the peer review and production processes to speed up the time to first decision and time to publication. Our turnaround time continues to be comparable or even better than many reputable journals with a mean submission to first decision time of 31 days.

At this juncture, I am planning to move for an extended period of time to another institution outside the country for academic reasons. With this move, I have to pass the baton to another colleague who will certainly lead the *Annals* to continue its noble message. Dr. Mohammed Ahmed is an outstanding clinician/scientist and an expert in endocrinology. I have no doubt that with his leadership and vision and with the support of a group of experts in the Editorial Board and the hard-working and competent editorial staff, the *Annals* will continue to flourish as the leading journal in this region of the world. I would like to take this opportunity to thank the authors, the reviewers, the editorial board and staff who all contributed to make the *Annals* a publication worth reading. It has been a wonderful challenge to be the Editor-in-Chief of the *Annals* and a pleasure to serve its readership.



Ali S. Alzahrani
Editor-in-Chief

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SUMMARIES

INFECTIOUS DISEASE

Pandemic influenza A H1N1 in Saudi Arabia: description of the first one hundred cases

Memish and colleagues collected information on cases of influenza caused by the pandemic H1N1 virus in Saudi Arabia between June 1 and July 3, 2009. Most (57%) of the cases occurred in patients younger than 30 years of age, 47% of cases were imported, and the most common nationalities were Saudi and Filipino. The most common symptoms were fever, cough and sore throat. The authors recommend comprehensive and effective measures to control spread of the virus.

Mother-to-child transmission of HIV: experience at a referral hospital in Saudi Arabia

Of 39 infants born to HIV positive mothers at a tertiary care center in Riyadh between January 1994 and June 2006, Edathodu and colleagues found that all tested negative to HIV. Previously, a high rate of mother-to-child transmission of HIV was reported for Saudi Arabia. All of the mothers were taking antiretroviral therapy during pregnancy and delivery.

SOLID ORGAN TRANSPLANTATION

Analysis of complications in hepatic right lobe living donors

Azzam and colleagues analyzed postoperative complications in right lobe liver donors from 311 liver transplantations performed between February 1998 and December 2003. Thirty percent of donors experienced one or more complications. Biliary complications were most common, followed by persistent abdominal fluid collection. Remnant liver size and anatomical variations in the biliary tree are important risk factors for postoperative complications.

DIABETES

Prevalence of elevated hepatic transaminases among Jordanian patients with type 2 diabetes mellitus

In 1014 type 2 diabetic patients, Judi and colleagues found elevated ALT in 10.4% and elevated AST in 5.4%. Male gender, large waist circumference, younger age and noninsulin use were associated with elevated ALT levels.

MEDICAL GENETICS AND CARDIOLOGY

Endothelial nitric oxide synthase gene Glu298Asp polymorphism in patients with coronary artery disease

Salimi and colleagues investigated the association between NOS3 gene polymorphisms and coronary artery disease by comparing 241 CAD patients with 261 control subjects without CAD. Genotype frequencies differed significantly between the groups. The Glu298Asp polymorphism was an independent risk factor for CAD.

The First Influenza Pandemic of the 21st Century

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The 2009 H1N1 influenza virus (formerly known as swine flu) first appeared in Mexico and the United States in March and April 2009 and has swept the globe with unprecedented speed as a result of airline travel. On June 11, 2009, the World Health Organization raised its pandemic level to the highest level, Phase 6, indicating widespread community transmission on at least two continents. The 2009 H1N1 virus contains a unique combination of gene segments from human, swine and avian influenza A viruses. Children and young adults appear to be the most affected, perhaps reflecting protection in the elderly owing to exposure to H1N1 strains before 1957. Most clinical disease is relatively mild but complications leading to hospitalization, with the need for intensive care, can occur, especially in very young children, during pregnancy, in morbid obesity, and in those with underlying medical conditions such as chronic lung and cardiac diseases, diabetes, and immunosuppression. Bacterial co-infection has played a significant role in fatal cases. The case of fatality has been estimated at around 0.4%. Mathematical modeling suggests that the effect of novel influenza virus can be reduced by immunization, but the question remains: can we produce enough H1N1 vaccine to beat the pandemic?

With the emergence of the second wave of the 2009 influenza A (H1N1) virus, there have been concerns that this pandemic may rival those of 1957, 1968, and even 1918 in which not thousands, but millions of people around the world died from the disease (Table 1).[1,2] WHO is advising countries of the northern and southern hemispheres to prepare for a second wave of H1N1, in which large numbers of severely ill patients requiring more and more intensive care infrastructure are likely to be seen, creating pressures that could overwhelm hospitals and intensive care units and possibly disrupt the provision of care of other diseases. The newly developed H1N1 vaccine is expected to reduce the impact of the second wave of H1N1 influenza in the population, especially on high-risk groups, with diminished complications, hospitalization rates and mortality. On the other hand, previous H1N1 strains have developed antiviral resistance, and this, as well as mutation to greater virulence, remain concerns for the future.[3,4] Past pandemics were characterized by several features that we have seen since March, 2009: the rapid spread of a virus with novel antigenic determinants; a change in pathogenicity with high death rates in younger age groups; successive pandemic waves; apparent higher transmissibility than that of the seasonal influenzas; and differences in impact in different geographic region.[5] The overall mortality in the previous century's three pandemics ranged from 1 million to more than 45 million deaths.[2,6] In the three previous influenza pandemics, vaccines were not produced in time to have any substantial impact.[7] Even though the technology of vaccine manufacture (production in embryonated eggs) has changed little since the 1930's, there is some hope that vaccines will be available to mitigate the force of later waves of the current epidemic. In addition, several clinically useful antiviral drugs are now available, although there are still concerns about development of resistance.[3]

Influenza virus: Back to basics

The viruses that cause influenza (influenza A, B, and C) belong to the family Orthomyxoviridae, which is char-

Table 1. Influenza pandemic of the 20th century.

Date	Strain	Estimate number of worldwide deaths	Comments
1918-1919 (Spanish Flu)	H1N1	Over 50 million	Three waves: A first, mild wave in the spring of 1918 was replaced by a second wave in September to November, 1918 that resulted in a mortality rate of over 2.5%. A third wave with equally high mortality rates swept around the world in 1919. The virus probably originated from the United States and then spread to Europe.
1957-1958 (Asian Flu)	H2N2	1-1.5 million	Two waves: The virus originated in Southern China in February 1957 and spread over 3 months to Singapore, Hong Kong and Japan and in October 1957 reached the United Kingdom and United States. A second wave was detected in January 1958.
1968-1969 (Hong Kong Flu)	H3N2	¾ million	Two waves in the winters of 1968-1969 and 1969-1970. The virus originated from Hong Kong in July 1968.

acterized by a segmented minus-strand RNA genome. Influenza A and B viruses genomes consist of 8 separate segments. These include the following: three transcriptases (PB1, PB2, and PA), two surface glycoproteins, the hemagglutinin (H or HA) and neuramidase (N and NA), two matrix proteins (M1 and M2), and one nucleocapsid protein (NP). Epidemic disease is caused by influenza viruses type A and B. Type C influenza viruses cause sporadic mild influenza-like illness in children. The focus of this article will be on influenza A virus, which may infect humans and birds and most importantly has the capability of developing into pandemic virus.[8] Type A influenza has been divided into multiple subtypes, and the natural host for most of these are various avian species. In addition, influenza A viruses of a few distinct subtypes have been isolated from pigs, horses, seals, whales and human beings. The genome of the virus codes for two important surface glycoproteins, the hemagglutinin (H or HA) and the neuraminidase (N or NA). Based on both sequence and antigenic analysis, sixteen distinct H (H1-H16) and nine distinct N (N1-N9) subtypes are now recognized in animal and avian influenza viruses, but only 3 H subtypes (H1, H2 and H3) and 2 N subtypes (N1, N2) have caused extensive outbreaks in human beings.[9] The influenza virus has a poor ability to proofread its genetic material while replicating, which results in frequent errors in progeny genes, and thus frequent mutations. When such minor changes occur in the H and N proteins they result in "antigenic drift," the slow but significant change in antigenicity that occurs over time in both influenza A and influenza B and that requires periodic changes in the yearly vaccine.[10] An example of such drift occurred during the 2003/2004 influenza season when the H3N2 circulating virus developed over 80% drift from the virus that was used to make one of the three major vaccine components that year (Table 2). Further, marked changes in H, with or without similar changes in N, termed "antigenic shift"[11] occur when new H or N gene segments are acquired by a process known as "reassortment." This may take place by the mixing of genetic segments during dual infection of cells by a human and an animal virus. When such viruses containing reassorted gene segments are introduced into a population that has no pre-existing immunity, they may lead to a pandemic. This happened in 1957 and 1968.[10,11]

Devastating pandemics take place when populations are exposed to a new viral subtype in the absence of pre-existing immunity. The infectious capabilities of a new virus that emerges in this way through reassortment are likely to be acquired from one or more of the human influenza gene segments. Conditions favorable for the emergence of an antigenic shift (reassortment) involve humans living in close proximity to domestic poultry and pigs.[12] Pigs play an important role in interspecies transmission of influenza virus. Susceptible pig cells process receptors for both avian and human influenza strains which allow the pigs to serve as mixing vessels for the exchange of genetic material between human and avian viruses resulting in the appearance of novel subtypes. Analysis of the 1957 H2N2 pandemic strain found that the emergent virus resulted from the acquisition by previously circulating human H1N1 of three new gene segments of avian origin (the H2 gene, the N2 gene, and one other). Similarly, the 1968 pandemic H3N2 virus acquired two new genes from an avian virus closely related to viruses isolated from ducks in Asia in 1963. In contrast, the 1918 H1N1 virus appears to have been an avian-like influenza virus derived in toto from an unknown source.[13,14] The currently circulating novel influenza H1N1 viruses that have been isolated around the globe during 2009 appear to have originated from two unrelated swine viruses, one of them a derivative of the 1918 human virus.[15]

Evolution, zoonotic transmission and possible origin of 2009 H1N1 (Swine influenza)

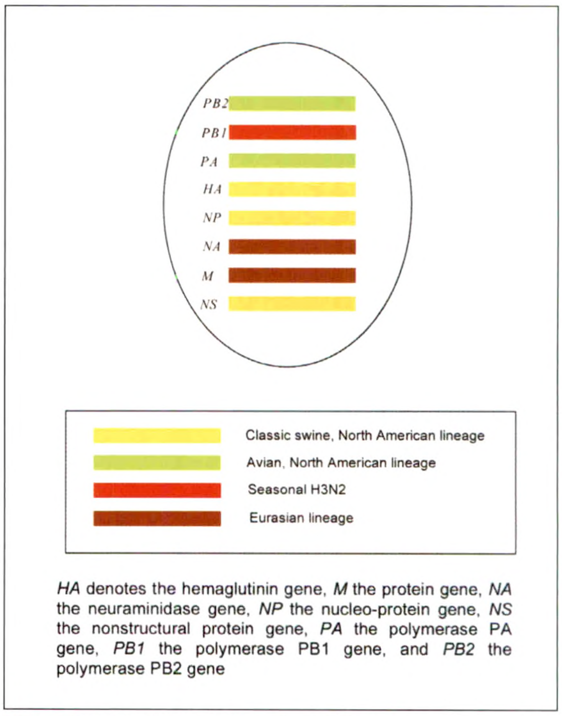
The 1918 H1N1 pandemic is believed to have also affected swine at that time. Its descendents have been enzootic in pigs up ever since (Table 3).[15,16] The first influenza A isolated from diseased pigs in the United States (USA) was in 1930.[17] These H1N1 swine viruses are called the classical swine H1N1 viruses and have continued to circulate in pigs in the Americas, Asia and, until 1980, also in Europe, and they remain relatively antigenically stable.[18] This swine H1N1 subtype has crossed over to humans periodically, including the Fort Dix outbreak in 1976,[19] resulting in infections that have been occasionally fatal, particularly in pregnant or immunocompromised persons, but not producing human epidemics. Moreover, following the human pandemic of the H3N2 subtype in 1968, H3N2 influenza virus infected pigs although such porcine strains have shown less antigenic drift in swine than in humans.[20] In 1998, H3N2 viruses with genes derived from human, swine and avian genes of North America ("triple reassortant viruses") were first isolated from pigs in the USA.[21] The triple reassortant H3N2 viruses also continue to acquire other virus genes via reassortment to generate triple reassortant H1N2 or H1N1 viruses.[22] Swine viruses of subtypes H1N1, H1N2 and H3N2 have been reported to cause occasional human infection during this time.[23] Between 1958 and 2005, 37 human swine-origin influenzas were reported. Twenty-two (51%) of these cases reported recent exposure to pigs. The overall fatality rate was 17%.[24] Prior to the current pandemic, but after December 2005, eleven sporadic cases of triple reassortant H1 viruses were reported to the Centers for Disease Control and Prevention (CDC) in the USA, ten carrying H1N1 genes and one H1N2 genes. Some of the patients had close exposure to pigs. Possible limited human-to-human transmission was reported in several situations.[25] Genetic analysis of 2009 H1N1 viruses isolated in North America, Europe and Asia revealed quadruple reassortant swine influenza A viruses that have not been recognized previously in pigs or humans. The virus resulted from the reassortment of North American H3N2 and H1N2 swine viruses (triple reassortment viruses: avian/swine/human with Eurasian swine viruses).[15,26-28]

Table 2. Antigenic drift and shift.

Drift	Shift
Minor change within subtype	Major change, new subtype
Point mutations	Exchange of gene segments
Occurs in A and B subtypes	Occurs in A subtypes only
May cause epidemics	May cause pandemic
Example: A/Fujian (H3N2) replaced A/Panama (H3N2) in 2003-2004	Example: H3N2 replaced H2N2 in 1968

Table 3. Evolution of swine influenza A virus.

1918-1919	H1N1 pandemic also affected swine
1930	The first isolation of H1N1 in pigs
1968	H3N2 infect swine in Asia after human pandemic
1976	Outbreak of new H1N1 swine strain of A/New Jersey/1976 occurred in military personnel at Fort Dix, New Jersey
1998	Triple reassortant viruses were isolated from pigs
1958-2005	37 human swine-origin influenza were reported
2005-2009	11 sporadic triple reassortant swine influenza viruses were reported in human
2009	New quadruple reassorted swine influenza H1N1 strain(A/California/07/2009)emerged in human populations and caused global influenza pandemic


PB2
PB1
PA
HA
NP
NA
M
NS
Classic swine, North American lineage
Avian, North American lineage
Seasonal H3N2
Eurasian lineage

HA denotes the hemaglutinin gene, *M* the protein gene, *NA* the neuraminidase gene, *NP* the nucleo-protein gene, *NS* the nonstructural protein gene, *PA* the polymerase PA gene, *PB1* the polymerase PB1 gene, and *PB2* the polymerase PB2 gene

Figure 1. 2009 influenza A (H1N1) virus genotype.

Sequence analysis also suggests that PB2 and PA genes originated from American H3N2 avian virus; a PB1 originated from H3N2: HA, NP, and NS genes originated from classical swine virus: and NA and M genes originated from Eurasian swine virus (Figure 1). One of swine genes of this new virus derived from the 1918 human virus, so the strain causing the 2009 pandemic is a fourth generation descendant of the 1918 virus.[15] The 2009 H1N1 viruses are more pathogenic in mammalian models than seasonal H1N1 viruses, showing the ability to replicate and cause appreciable pathology in the lungs of mice, ferrets and non-human primates. The pathologic changes seen were similar to those found in the lungs of animals infected with the highly pathogenic H5N1 avian influenza virus.[28]

Epidemiology and impact

Epidemiological data now indicate that the 2009 H1N1 influenza virus pandemic started as an outbreak of influenza like illness in the Mexican town of La Gloria, Veracruz, in mid-February 2009. In mid-April the Center of Disease Control (CDC) identified swine origin H1N1 influenza virus in two specimens independently collected in southern California. By the end of April, international spread and human-to-human transmission prompted the WHO to increase the pandemic alert from Phase 3 to Phase 4 and shortly after to Phase 5. On June 11, 2009, the WHO raised its pandemic to the highest level Phase 6, indicating widespread community transmission on at least two continents (Table 4).[30-35] As of December 27, 2009, more than 208 countries and overseas territories/communities have each reported at least one laboratory-confirmed case of pandemic H1N1 influenza, with a total or more than 622 000 laboratory confirmed cases and at least 12 220 deaths. However, the number of cases reported vastly underestimates the real number of cases: the WHO ceased regular reporting of case counts on July 16, 2009 because many countries were having difficulty tracking their numbers, and the WHO judged that their time would be better spent on investigating severe cases and other exceptional events. Most patients in the world with 2009 H1N1 have been teenagers and young adults, with rates of hospitalization highest in very young children. Between 1% and 10% of clinical illness require hospitalization. Overall from 7% to10% of all hospitalized patients are pregnant women in their second or third trimester. Of the hospitalized patients from 10% to 25% have required admission to intensive care, and 2% to 9% have died.[36] Little is known about the level of pre-existing immunity to the 2009 H1N1 virus. Recent studies suggest that persons under the age of 30 years have little evidence of protective antibodies. However, a portion of older adults have pre-existing cross-reactive antibodies, presumably as a result of exposure to H1N1 strains circulating before 1957.[37,38] Transmission of 2009 H1N1 virus from person to person is similar to that of other influenza viruses. The main route of transmission is respiratory through inhalation of large-particle respiratory droplets, and possibly via droplet nuclei. Transmission via large-particle droplets requires close contact because these droplets do not remain suspended in the air and generally travel only short distances (less than 2 meters). Contact with contaminated surfaces is another possible source of transmission. All respiratory secretions and bodily fluids (e.g. fomites, diarrheal stool) of infected persons should be considered potentially infectious.[39-41] The secondary attacks rate in households was estimated to be 27.3% and in school settings an infected school child was estimated to infect 2.4 other children within the school.[39] The estimated incubation period could range from 1 to 7 days, but is most likely 1 to 4 days. Infected persons can be assumed to be shedding virus from one day prior to illness onset until resolution of symptoms (up to 7 days following illness onset). Children and immunocompromised or

Table 4. World Health Organization pandemic levels.

Phase 1	No viruses circulating among animals have been reported to cause infections in humans.
Phase 2	An animal influenza virus circulating among domesticated or wild animals is known to have caused infection in humans, and is therefore considered a potential pandemic threat.
Phase 3	An animal or human-animal influenza reassortant virus has caused sporadic cases or small clusters of disease in people, but has not resulted in human-to-human transmission sufficient to sustain community-level outbreaks. Limited human-to-human transmission may occur when there is close contact between an infected person and an unprotected caregiver, but the virus is not widely transmitted among humans.
Phase 4	Verified human-to-human transmission of an animal or human-animal influenza reassortant virus able to cause" community-level outbreaks". The risk of pandemic is significantly raised.
Phase 5	Human-to-human spread of the virus into at least two countries in one WHO region. The declaration of Phase 5 is a strong signal that a pandemic is imminent
Phase 6	The pandemic phase is characterized by community level outbreaks in at least one other country in a different WHO region in addition to the criteria defined in Phase 5. A global pandemic is under way.

immunosuppressed persons may be contagious for longer periods. The amount of virus shed is greatest during the first 2 to 3 days of infection and appears to correlate directly with the height of fever.[42] The 2009 pandemic H1N1 virus is expected to come in waves, and we are now in the middle of the second wave. This wave may continue during winter, or there may be a third wave.[43] As of today no increase of severity has been seen and genetic mutations have been minimal.

Clinical features

The clinical manifestations can vary from asymptomatic infection to serious fatal illness that may include exacerbation of other underlying conditions or severe viral pneumonia with multi-organ failure. The Centers for Disease Control and Prevention (CDC) defines cases as influenza-like illness (ILI) if there is a fever of >37.8°C (>100°F) plus cough and/or sore throat in the absence of a known cause other than influenza. In the outbreak of 2009 H1N1 influenza pandemic in New York City, 95% of virologically proven cases satisfied the ILI definition.[40] Fever has been absent in some outpatients and in up to 1 in 6 surviving hospitalized patient.[44] Vomiting and or diarrhea have occurred in up to 38% of outpatients in United States.[32,44] Young children may have atypical influenza illness with absence of fever and cough. Among 89 children with confirmed H1N1 who required hospitalization in Birmingham, UK, the most common symptoms were fever (81%), cough (73%), and diarrhea (62%).[45] Infants may present with fever and lethargy. The CDC case definitions for confirmed, probable and suspected cases are presented in Table 5.

Three categories of clinical presentations have been seen during the current pandemic:[40,46]

1. Mild illness characterized by fever (some patients had no fever), cough, sore throat, diarrhea, myalgias, headache. Other frequent findings have included chills and malaise. Vomiting and diarrhea have been reported in some patients, but no shortness of breath, dyspnea, or severe dehydration.
2. Progressive illness characterized by mild illness in addition to signs or symptoms suggesting a progressive illness, which include (Table 6):
   a. Chest pain, tachypnea, or labored breathing in children
   b. Hypotension
   c. Confusion or altered mental status
   d. Severe dehydration or exacerbations of a chronic conditions (e.g. asthma, cardiovascular conditions)
3. Severe illness characterized by the following:
   a. Profound hypoxemia, abnormal chest radio graph, and mechanical ventilation
   b. Encephalitis or encephalopathy
   c. Shock, multisystem organ failure
   d. Myocarditis and rhabdomyolysis
   e. Invasive secondary bacterial infection (e.g. pneumococcal disease)

Table 5. CDC: Case definition for 2009 H1N1 influenza virus.

Confirmed case:	An individual with an acute febrile respiratory illness with laboratory confirmed 2009 H1N1 infection by one or more of the following tests: - Real time reverse-transcription polymerase (rRT-PCR), or - Viral culture
Probable case	An individual with influenza like illness (i.e. an illness with a fever and cough or sore throat) who is positive for influenza A, but negative for H1 and H3 by rRT-PCR
Suspected case	An individual who does not meet the definitions of confirmed or probable pandemic H1N1 influenza A, but has ILI an epidemiologic link (e.g. likely exposure to a confirmed or probable case within the past 7 days.

Complications

Most patients appear to have mild illness and recover spontaneously. Approximately 2% to 5% of laboratory-confirmed 2009 A (H1N1) influenza in Canada and in the United States as well as 8% in Mexico have required hospitalization.[44] Nearly three-quarters of cases in the USA requiring hospitalization, as well as 21 (46%) of 45 fatal cases in Mexico, involved one or more underlying conditions including asthma, diabetes, heart or lung disease, neurologic disease, pregnancy, morbid obesity, autoimmune disorders and associated immunosuppressive therapies.[44,46,47] Forty-five percent of patients admitted to intensive care units in the USA series were children under the age of 18 years, and 5% were 65 years of age or older. Surveillance of pediatric deaths reported by CDC indicated that, of 36 children who died, seven (19%) were aged <5 years, and 24 (67%) had one or more high-risk medical conditions. Twenty-two (92%) of the 24 children with high-risk medical conditions had neurodevelopmental disabilities which included cerebral palsy, developmental delay, autism, congenital neurological disorders and other central nervous system disorders.[49] Pneumonia is the most common and serious complication of the 2009 H1N1 pandemic influenza. The clinical course of 45 fatal cases in Mexico was characterized by severe pneumonia, hypoxemia with multifocal infiltrates including nodular alveolar, or basilar opacities on chest x-ray, and rapid progression to acute respiratory distress syndrome (ARDS) and renal or multi-organ failure. A similar experience was re-

Table 6. Clinical signs indicating rapid progression and need for urgent medical care.

In adults	In children
- Difficult breathing or shortness of breath	- Tachypnea or labored breathing
- Pain or pressure in the chest or abdomen	- Skin color change, gray or blue
- Episodes of sudden dizziness	- Inadequate intake of oral fluids
- Severe or continuous vomiting	- Severe or continuous vomiting
- Influenza-like illness that improves but then returns with fever and cough	- Influenza-like illness that improves but then returns with fever and cough
- Confusion	- Irritable, or not waking up

ported from Canada, Australia, and the New Zealand. Some patients who required intensive care required advanced mechanical ventilation with high-frequency oscillatory bilevel ventilation and mean airway pressures of 32 to 55 cm/H_2O or veno-venous extracorporeal membrane oxygenation (ECMO) support.[50-54] Bacterial co-infections likely played a role in almost one-third of fatal cases of 2009 pandemic influenza A (H1N1) in the USA. The CDC investigators found evidence of concurrent bacterial infection in lung specimens from 22 of 77 patients (29%) with fatal pandemic H1N1 infection. A total of 10 cases were co-infections with *Streptococcus pneumoniae*, 6 with *Streptococcus pyogenes*, 7 with *Staphylococcus aureus*, 2 with *Streptococcus mitis* and 1 with *Hemophilus influenza*. Four of the fatal cases involved multiple pathogens. The age of patients ranged from 2 months to 56 years, with a median of 31 years.[55] Among other complications of pandemic H1N1 are acute neurologic syndromes reported in four patients aged 7 to 17 years who were admitted with signs of ILI and findings that included seizures or altered mental status in 2 children, encephalitis in 2, and ataxia in 1. Three of the four patients had abnormal electroencephalogram (EEG). In all patients pandemic H1N1 viral RNA was detected in nasopharyngeal specimens but not in cerebrospinal fluids (CSF). All recovered without sequelae.[56] The overall case-fatality rate was 0.4% (compared with 2.4% for the 1918-1918 influenza pandemic) based on surveillance data from Mexico and mathematical modeling.[57,58] There was a documented underlying medical condition in at least 49% of global documented fatal cases.[58]

Diagnosis

When influenza viruses are known to be circulating in the community, patients presenting with mild influenza can be diagnosed on clinical and epidemiological grounds alone. All patients should be instructed to return for follow-up should they develop any signs or symptoms of progressive disease (Table 6) or fail to improve within 72 hours of the onset of symptoms. Under no circumstances should influenza diagnostic tests delay initiation of infection control practices or antiviral treatment if 2009 H1N1 pandemic disease is suspected. Laboratory testing should be prioritized to include hospitalized patients; patients where a diagnosis of influenza will inform decisions regarding clinical care, infection control, or management of close contacts; and patients who have died of an acute illness in which influenza was suspected.

The gold standard for laboratory diagnosis of the 2009 H1N1 influenza is the real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test, using primer and detector sequences tailored to the specific detection of this virus. A number of other diagnostic tests are available to detect the presence of 2009 H1N1 influenza in clinical specimens, but they differ in their sensitivity and specificity. Rapid influenza diagnostic tests are based on various forms of antigen detection and have high specificity (>95%) but variable sensitivity (10-70%).[59-60] Preferred respiratory specimens include a nasopharyngeal swab with synthetic tip (e.g. polyester or dacron), nasal wash, bronchoalveolar lavage (BAL) or endotracheal aspirate. Lower respiratory tract specimens have a higher yield in patients with pneumonia due to viral replication in the lower respiratory tract. Many experts advise the use of a combination of nasopharyngeal swab with oropharyngeal swab. Isolation of H1N1 virus in cell culture or embryonated eggs is diagnostic for infection, but it may not a yield timely results for clinical management; in addition a negative viral culture does not exclude infection.[59,60] All diagnostic laboratory work on clinical sample from patients who are suspected cases of influenza H1N1 virus infection should be done in a biosafety level 2 (BSL-2) laboratory. Growth of H1N1 virus in cell culture or embryonated eggs should be performed in a BSL-2 laboratory using BSL-3 practices.[60]

Management of 2009 H1N1 influenza

The majority of individuals infected with the pandemic H1N1 influenza A virus can be treated with simple supportive care at home using antipyretics (e.g. acetaminophen or ibuprofen). Aspirin (acetylsalicylic acid) or aspirin-containing products (e.g. bismuth subsalicylate, Pepto-Bismol) should not be used in children <18 years due to the risk of Reye's syndrome.

Empiric antiviral therapy should be started as soon as possible for persons with suspected probable or confirmed influenza and:[45]

Table 7. High risk groups for severe illness.

Children younger than 2 years old
Pregnant woman up to 2 weeks post partum (regardless how the pregnancy ended)
Adult, 65 years of age or older
Persons younger than 19 years who are receiving long-term aspirin therapy.
Persons with medical condition including asthma, neurological and neurodevelopmental conditions (including disorder of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy) chronic obstructive lung disease, cardiac disease, diabetes mellitus, immunosuppressive conditions (including HIV/AIDS, and cancer)

Table 8. Antiviral treatment and chemoprophylaxis of 2009 H1N1 influenza.

Medication/Age groups		Treatment (5 days)	Chemoprophylaxis (10 days)
Oseltamivir			
Adults		75 mg twice daily	75 mg once per day
Children (age≥12 months), weight	≤15 kg	30 mg twice daily	30 mg once per day
	15-23 kg	45 mg twice daily	45 mg once per day
	24-40 kg	60 mg twice daily	60 mg once per day
	>40 kg	75 mg twice daily	75 mg once per day
Children	Age 3 months to <12 months	3 mg/kg/dose twice daily	3 mg/kg/dose once per day
Children	0-<3 months	3 mg/kg/dose twice daily	Not recommended, unless situation judged critical (limited data)
Zanamivir			
Adults		Two 5-mg inhalations (10 mg total) twice daily	Two 5-mg inhalations (10 mg total) once daily
Children	≥7 years or older for treatment; ≥5 years for chemoprophylaxis	Two 5-mg inhalations (10 mg total) twice daily	Two 5-mg inhalations (10 mg total) once daily

1. Illness requiring hospitalization
2. Progressive, severe or complicated illness regardless of previous health status and/or
3. High risk for severe disease (Table 7)

Recent reports have shown that 21% to 25% of hospitalized patients with confirmed 2009 H1N1 infections have not received antivirals or have delay in receiving antivirals.[47,48] Among 27 fatal cases in Mexico, the median time from the appearance of symptoms to treatment with antivirals was 8 days (range, 1-26 days).[44]

Antiviral drugs for treatment of 2009 H1N1 influenza (Table 8)

The neuraminidase inhibitors, oseltamivir (Tamiflu) and zanamivir (Relenza) are the drugs of choice for treatment and while the vast majority of pandemic H1N1 circulating strains are sensitive to these medications, all strains tested are resistant to amantadine and rimantadine.[61,62]

Oseltamivir and zanamivir are generally well-tolerated. Nausea and vomiting were reported with moderate frequency among adults receiving oseltamivir for treatment (nausea without vomiting, 10%; vomiting 9%). In children treated with oseltamivir, 14% reported vomiting. Oseltamivir suspension is formulated with sorbitol, which may be associated with diarrhea and abdominal pain in patients who are fructose-intolerant. Zanamivir is formulated for oral inhalation and is contraindicated in patients with asthma or chronic obstructive disease. As of December 18, 2009, 136 isolates (among more than ten thousand tested) of pandemic H1N1 were resistant to oseltamivir.[62] Among the 32 cases for whom detailed information was available, 16 were associated

Table 9. ACIP priority target groups for H1N1 influenza vaccine

Pregnant woman
Household contact and caregivers for infant younger than 6 months of age
Health-care and emergency medicine personnel
All people from 6 months through 24 years of age
Persons aged 25 through 64 years who have health conditions associated with high risk of medical complications from influenza (Table 7)

with antiviral prophylaxis, and three had no history of exposure to oseltamivir. Resistance was associated with the common H275Y mutation with retention of zanamivir susceptibility.[61] Antiviral therapy is most effective when started within 48 hours after the onset of symptoms; however, evidence suggests that treatment[62] may benefit patients with prolonged or severe illness even when started more than 48 hours after the onset of illness. The recommended duration of treatment is 5 days. Hospitalized patients with severe infection might require longer antiviral courses. Some experts have advocated use of doubled doses of oseltamivir in critically ill patient despite lack of published date about efficacy. Zanavimir inhaled formulation is not designed to be used in any nebulizer or mechanical ventilator[62,63] as there is a risk that the lactose drug carrier can obstruct ventilator equipment. For patients who are unable to take oral medication or in whom oral medication appears to be ineffective, peramavir, an investigational neuraminidase inhibitor formulated for intravenous administration, can be requested from the CDC under Food and Drug Administration (FDA) and emergency use authorization, although studies on efficacy and safety are limited.[62,63]

Symptomatic patients who have highly suspected or documented oseltamivir resistance should not be treated with peramivir because strains with the H275Y mutation have demonstrated reduced in vitro susceptibility to peramivir. These patients should be treated with intravenous zanamivir, which is an investigational drug that can be requested from FDA for compassionate use.[62,63] The CDC suggests limiting the use of antiviral chemoprophylaxis to specific groups. Antiviral doses recommended for treatment and prophylaxis of 2009 H1N1 influenza in adult and children are listed in Table 8. Clinicians should consider empiric treatment with antibacterial drugs if bacterial co-infections is suspected during or after influenza. Antibiotic selection should take into consideration local data regarding frequency of pathogens causing secondary infection and the pattern of drug resistance. When pneumonia is present, treatment with antibiotics should follow evidence-based guidelines for community-acquired pneumonia.

The use of corticosteroids for H1N1 influenza is controversial. High-dose systemic corticosteroids are not recommended for use in viral penumonitis outside clinical trials. However, low-dose steroids may be considered in patient with septic shock who require vasopressors.[44, 64, 65]

Isolation of the hospitalized patient with 2009 H1N1 infection

CDC recommends standard, droplet, and contact precautions for care of patients with suspected or confirmed 2009 H1N1 influenza infection. Health care workers should use surgical masks for routine non-aerosolizing patient care and N95-respirators for aerosol-generating procedures. Isolation precautions should continue for 7 days after illness onset or until 24 hours after the resolution of fever and respiratory symptoms. A longer period of isolation may be considered in the case of young children and severely immunocompromised patients.[60,66]

2009 H1N1 vaccine

An effective vaccine is the best tool to prevent the unpredictable spread of the current influenza pandemic. The 2009 H1N1 virus has the potential to cause severe disease, death, and potential socioeconomic dysfunction, and mathematical modeling suggests that the effect of the virus can be reduced by immunization.[67,68] Two types of H1N1 vaccines which have been prepared and have received approval from the FDA or the European Medicine Agency (EMEA) for use in the prevention of influenza caused by the 2009 pandemic influenza A (H1N1) virus. Both adjuvanted and unadjuvanted vaccine formulations are available. An adjuvant is a substance that boosts the immune response. It is made up of naturally occurring oil, water and vitamin E. The unadjuvanted vaccine does not include this material. Vaccination campaigns are currently underway to protect populations from pandemic H1N1. Preliminary data indicate that both vaccines are safe and immunogenic.[69,71-73] The Advisory Committee on

Immunization Practice (ACIP) recommends that vaccination efforts should focus initially on persons in five target groups at high risk for influenza-related complications (Table 9).[70]

People who have had egg allergies should not receive the H1N1 vaccine without first consultation a physician because the viruses for the vaccine are grown in chicken-egg-based cultures. On November 19, 2009, the WHO estimated that around 80 million doses of pandemic vaccine had been distributed globally and around 65 million people had been vaccinated. The side-effect profile of the H1N1 vaccine (adjuvanted and unadjuvanted) particularly the frequency and severity of solicited adverse events is consistent with previous experience with seasonal influenza vaccine. To date, less than ten suspected cases of Guillain-Barré syndrome have been reported in people who have received vaccines. These numbers are in line with normal background rates of this illness as recently reported. All such cases are been investigated to determine whether these are randomly occurring events or whether they might be associated with vaccination.[69] WHO has received no reports of fatal outcome or confirmed cases of Guillain-Barré syndrome since the H1N1 vaccination campaigns began. All cases have recovered. Intense active monitoring for rare adverse reactions of H1N1 vaccine is ongoing, but all data compiled to date indicate that pandemic H1N1 vaccines match the excellent safety profile of the seasonal influenza vaccines that has been used for more than 60 years.[69,70]

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original article

Pandemic influenza A (H1N1) in Saudi Arabia: description of the first one hundred cases

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BACKGROUND AND OBJECTIVES: In April 2009, the World Health Organization (WHO) declared pandemic influenza A (H1N1) "public health emergency of international concern". On June 11, 2009, WHO raised the pandemic alert level to phase 6, indicating a global pandemic. By December 2009, more than 208 countries and territories had reported swine flu cases. The descriptive epidemiology of the first reported 100 cases of this virus in Saudi Arabia are summarized in this report.

METHODS: Data were collected from 1 June to 3 July, 2009 using a predesigned questionnaire. Questionnaires were filled by Field Epidemiology Training Program residents. Data for the first 100 complete cases of confirmed pandemic influenza A (H1N1) were compiled and analyzed.

RESULTS: The age of reported cases was in the range of 1 to 56 years. The highest percentage of cases was in the age group of 20 to 30 years followed by the age group of 1 to 10 years. Females represented 55% of the cases; imported cases represented 47%, 58% of whom had come via the King Khaled Airport. The most common nationalities most were from Saudi Arabia and the Philippines. The main symptoms were fever (56%), cough (54%), and sore throat and the number of cases was seen to peak from the 27 to 29 June.

CONCLUSION: Pandemic influenza A (H1N1) is still a threat to Saudi Arabia. Thus, comprehensive and effective measures for surveillance and prevention of the disease are needed to control its spread.

Swine influenza during the 1918 flu pandemic infected one-third of the world's population (an estimated 500 million people) and caused approximately 50 million deaths.[1] In 1976, an outbreak of swine influenza occurred in New Jersey, USA, which involved more than 200 cases, some of them severe, resulting in one death.[2] In 1988, another fatality was reported as a complication of swine influenza. From 2005 until January 2009, 12 human cases of swine flu were reported in the USA, but none were fatal.[3] In 2009, cases of influenza-like illness were first reported in Mexico on March 18; the outbreak was subsequently confirmed as pandemic influenza A (H1N1).[4] Until early May, nearly 600 pandemic influenza A (H1N1) cases had been confirmed in Mexico, including 25 deaths.[5]

In late April, the WHO declared a "public health emergency of international concern" under the rules of the WHO's new International Health Regulations when the first few cases of the pandemic influenza A (H1N1) virus were reported in the United States.[6,7] As of late June, the WHO reported that pandemic influenza A (H1N1) had been confirmed in almost 60 000 people in more than 100 countries, and 263 deaths were confirmed to have been caused by the disease. On June 11, 2009, the WHO raised the pandemic alert level to phase 6 (indicating a global pandemic). By December 2009, more than 208 countries and territories had reported swine flu cases.[8]

Symptoms of the 2009 "swine flu" pandemic influenza A (H1N1) virus in humans are similar to those of seasonal influenza and of influenza-like illness in general. They include fever, cough, sore throat, body aches, headache, chills, and fatigue. However, the 2009 outbreak has shown an increased percentage of patients reporting diarrhea and vomiting.[9] In children, signs of severe disease include apnea, tachypnea, dyspnea, cyanosis, dehydration, altered mental status, and extreme irritability.[10] Pandemic influenza A (H1N1) (swine flu) tends to cause high morbidity but low mortality rates (1-4%). The most common cause of death is respiratory

Table 1. Distribution of cases by demographic data.

Variables	Frequency	Percent
Gender		
Male	45	45.0
Female	55	55.0
Total	**100**	**100.0**
Age group (years)		
1-9	22	22.0
10-19	11	11.0
20-29	35	35.0
30-39	19	19.0
40-49	5	5.0
50-59	8	8.0
Mean (SD)	24.2 (14.4)	
Total	**100**	**100.0%**

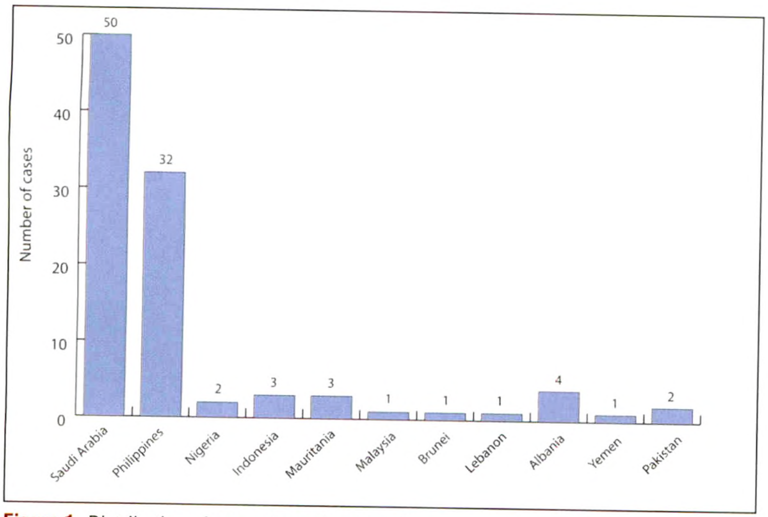

Figure 1. Distribution of cases by national origin.

failure; other causes of death are pneumonia, high fever leading to neurological problems, dehydration, and electrolyte imbalance. Fatalities are more likely in young children and the elderly.[11]

According to the Saudi Ministry of Health, the number of laboratory-confirmed cases in Saudi Arabia as of December 30, 2009 was 15850, with 124 deaths. This report summarizes the descriptive epidemiology of the first reported 100 cases of this virus in Saudi Arabia.

METHODS

Data were collected using a predesigned questionnaire. The first 114 confirmed pandemic influenza A (H1N1) cases were identified from the Ministry of Health (MOH) Infectious Diseases Department database during the period ranging from June 1 to July 3, 2009. Questionnaires with cover letters were sent to the designated hospitals for data collection. Patient medical records were the source of data. Questionnaires were filled by Field Epidemiology Training Program (FETP) residents in collaboration with medical colleagues from those hospitals. Data for the first 100 complete cases of confirmed pandemic influenza A (H1N1) were compiled and analyzed using the Epi Info program. None of the first 100 cases were severe cases and there were no deaths.

RESULTS

We were able to complete a questionnaire for the first 100 cases of pandemic influenza A (H1N1) from different hospitals. The age of the cases ranged between 1 and 56 years with a mean (SD) of 24.2 (14.4) years. The highest percentage of cases were in the age group of 20 to 30 years (35%) followed by the age group of 1 to 10 years (22%). There were 45 males and 55 females (Table 1). Fifty-three percent of the cases had some history of contact with diseased persons inside Saudi Arabia while 47% had traveled into Saudi Arabia. The most common national origins were Saudi Arabia and the Philippines (Figure 1).

The symptoms reported by the cases were fever (56%), cough (54%), sore throat (32%), rhinitis (17%), and difficulty breathing (8%) (Figure 2). The peak in the number of cases appeared between June 27 and 29. (Figure 3). Fifty-eight percent of the cases had entered the country through King Khalid Airport in Riyadh, 16% through King Fahad Airport in the Eastern region, and 14% through King Abdul Aziz Airport in Jeddah (Figure 4).

DISCUSSION

As of September 27, 2009, there have been more than 340 000 laboratory-confirmed cases of pandemic influenza A H1N1 2009 worldwide and over 4100 deaths have been reported to the WHO. As many countries have stopped counting individual cases, particularly the milder illnesses, the case count is significantly lower than the actual number of cases that have occurred.[12] Health authorities in Saudi Arabia kept a high level of alertness in monitoring the situation of pandemic influenza A (H1N1) all over the world especially during winter and around the Hajj season which prevented further spread of the virus locally, regionally, and internationally.

The first 100 cases of pandemic influenza A (H1N1) were mostly among youth aged less than 40 years. A characteristic feature of the pandemic influenza A (H1N1) is that it has mostly involved children and young adults. Similar results were observed in different parts of the world: in Europe, the age of confirmed cases ranged between 1 and 53 years with a median of 23 years.[13] One of the early studies from the USA showed that although the age of pandemic influenza A (H1N1) patients in the study ranged from 3 months to 81 years, 60% of patients were 18 years of age or younger. In most countries, the majority of Pandemic influenza A (H1N1) cases have been occurring in young people, with the median age estimated to be 12 to 17 years in Canada, the USA, Chile, Japan, and the UK. This age distribution speaks in favor of at least partial immunity to the virus in the older population.[14]

Among the cases in the present study, the main symptoms were fever, cough, and sore throat. H1N1 is a rather mild, self-limiting, upper respiratory tract illness with (or at times without) fever, cough, and sore throat. Up to 50% of all patients present with gastrointestinal symptoms including diarrhea and vomiting. The spectrum of clinical presentation varies from asymptomatic cases to primary viral pneumonia resulting in respiratory failure, acute respiratory distress, multiorgan failure, and death.[15] The rate of hospitalization could actually be as high as 10% in some cities. Most, but not all, of the hospitalized patients have underlying conditions such as cardiovascular disease, respiratory disease including asthma, autoimmune disorders, obesity, diabetes, or cancer. Pregnant women, especially in their second and third trimester, are also at a high risk for more severe disease.[16]

Nearly one half of all confirmed cases were found to have traveled into Saudi Arabia because of the large number of expatriate workers in different sectors. The country also annually hosts the largest international gathering of the Hajj where 2 to 3 millions gather in a small geographical area. This puts Saudi Arabia in the front line in facing the threat of pandemic influenza A (H1N1). Control measures will not only benefit Saudi Arabia, but will also be of benefit to other countries, especially those with low prevalence, to stop or control the spread of the epidemic.[17,18]

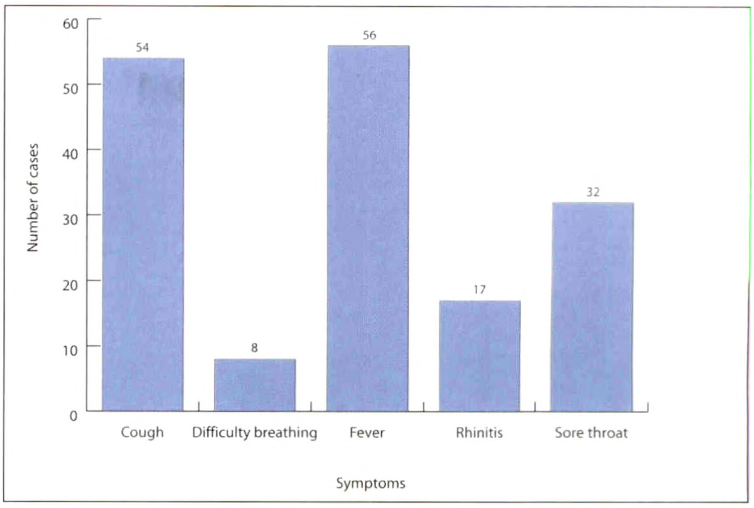

Figure 2. Distribution of cases by reported symptoms.

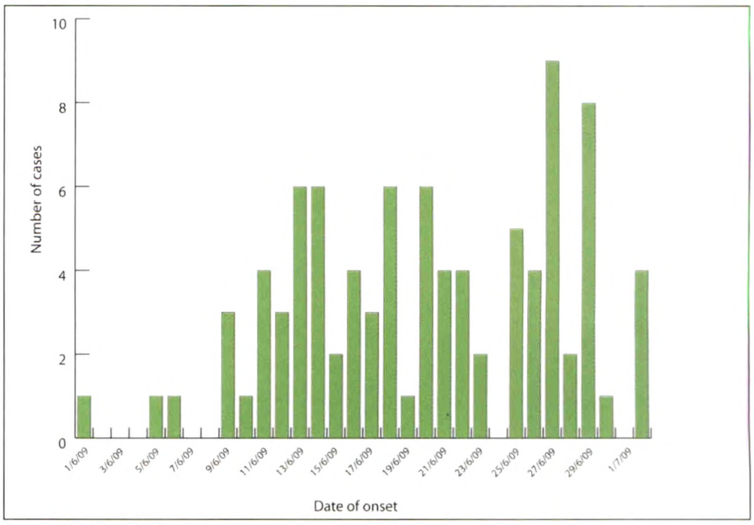

Figure 3. Distribution of cases by date of onset.

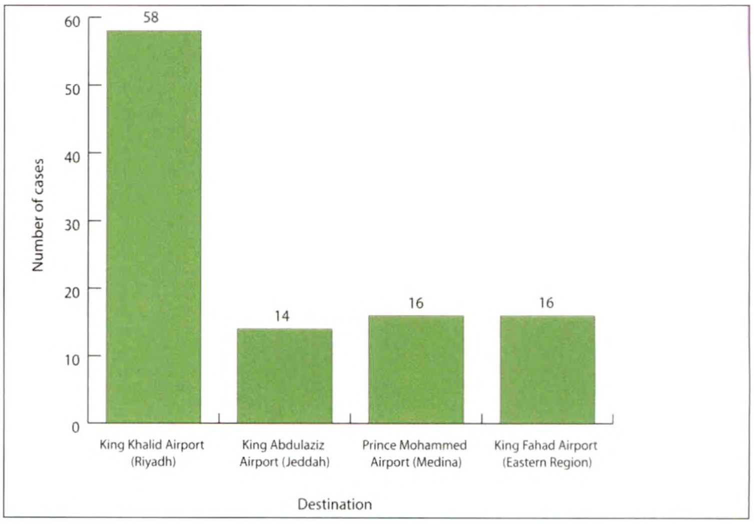

Figure 4. Distribution of cases by arrival destination.

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original article

Mother-to-child transmission of HIV: experience at a referral hospital in Saudi Arabia

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BACKGROUND AND OBJECTIVES: The rate of mother-to-child transmission of human immunodeficiency virus (HIV) type 1 has been reported to be high in Saudi Arabia. We report the rate of such transmission among a cohort of HIV-infected women enrolled in an HIV program at a tertiary care facility in Riyadh.

METHODS: All HIV-infected women who became pregnant and delivered during their follow-up between January 1994 and June 2006 were included in this study. HIV viral load and CD4+ T-lymphocyte count near-term, the mode of delivery, and the HIV status of the newborn at 18 months were recorded. All women were counseled and managed according to the three-step PACTG 076 protocol.

RESULTS: Of 68 HIV-infected women in the cohort, 31 had 40 pregnancies; one aborted at 13 weeks gestation. The mode of delivery was elective cesarean delivery in 28 pregnancies (70%) at 36 weeks gestation, and 11 (27.5%) had normal spontaneous vaginal delivery. The median CD4+ T-lymphocyte count near-term was 536 cells per cubic millimeter and the median viral load for 25 pregnancies was 1646 copies/mL, with only nine pregnancies (22.5%) having viral loads of more than 1000 copies/mL. Fourteen pregnancies (35%) had undetectable HIV prior to delivery. All patients were taking antiretroviral therapy during pregnancy and delivery. All 39 newborns tested negative for HIV infection at the age of 18 months; none of the newborns was breastfed.

CONCLUSIONS: Contrary to previous local experience, diagnosis, management, and antiretroviral therapy almost eliminated mother-to-child transmission of HIV-1 in our patient population.

Mother-to-child transmission (MTCT) of the human immunodeficiency virus (HIV) was noted very early in the history of acquired immune deficiency syndrome (AIDS).[1,2] As the epidemic of HIV/AIDS grew, women constituted 50% of infected people, and up to 60% in certain developing countries.[2] Subsequently, MTCT now is the cause of almost all pediatric HIV infections.[2] There is clinical and laboratory evidence to support several possible mechanisms for MTCT, including maternal disease state and viral load, fetal exposure to infected maternal body fluids during gestation and delivery, as well as breastfeeding.[3-8] There is now sufficient evidence to suggest that transmission during late pregnancy and the intrapartum period contributes relatively more to the overall rate of vertical transmission than during the early intrauterine period.[6] In the absence of any intervention, the rate of MTCT is reported to be 15% to 30 %.[8] The Pediatric AIDS Clinical Trials Group (PACTG) Protocol 076 demonstrated that a three-part regimen that included zidovudine, another nucleoside reverse transcriptase inhibitor and a protease inhibitor could reduce the risk of MTCT of the HIV virus by nearly 70%.[7,9]

Data on the epidemiology of HIV-1 infection from the Middle East are limited. An 18-year surveillance of the epidemiology of HIV/AIDS in Saudi Arabia showed 1285 citizens to be infected with the HIV virus: 26.5% of them were women, mostly in the reproductive age group; 12.1% were children younger than 14 years old, and more than 53% of infected children had acquired the virus from their mothers.[10] In another report from Riyadh, perinatal transmission accounted for 63% of all HIV infections in children.[11] Reports on pregnancy outcome in women who are HIV-infected from the region and from Saudi Arabia are limited. We present the data on pregnancy outcome for HIV-infected women who delivered at our facility.

METHODS

King Faisal Specialist Hospital and Research Centre (KFSHRC) is one of the referral centers for HIV-infected individuals in Saudi Arabia. The HIV Service of the Section of Infectious Diseases follows more than 500 HIV-infected adults. The screening test was done using AxSYM® HIV 1/2 gO MEIA (Abbott Laboratories, Abbott Park, IL, USA), is used to confirm the diagnosis of HIV infection. Abbot real-time PCR assay (Abbott RealTime M2000 rt, Abbott Laboratories, Abbott Park, Illinois, USA) is used used for the quantitation of HIV-1 virus. The lower limit of HIV viral load with this assay is 40 copies/mL. CD4+ count is done by standard flow cytometry using FACSCalibur™ (Becton Dickinson, San Jose, CA, USA).

Data were available for 102 HIV-infected women who were registered in the clinic. Sixty-eight were married, 13 were single, 17 were widowed, and 4 were divorced at the time of the study. Women older than 55 years of age, and pregnancies and deliveries that occurred before referral to our institution were excluded from the study. Data on demographics, number of pregnancies between January 1994 and June 2006, abortions, and mode of delivery were collected. Information was obtained about the use of zidovudine, viral load, and CD4+ T-lymphocyte count at about 36 weeks of gestation. Charts of the newborn were reviewed to collect data on the results of HIV antibody screening and HIV PCR at 18 months of age.

All women included in this study had been managed according to the three-step PACTG 076 protocol.[9] The antenatal component consisted of 300 mg zidovudine orally twice daily, initiated between 14 and 34 weeks gestation (if not already on antiretroviral therapy) in addition to another nucleoside reverse transcriptase inhibitor (lamivudine in most cases) and a protease inhibitor, either nelfinavir or lopinavir/ritonavir, which were continued throughout the pregnancy. Women already on antiretroviral therapy for HIV infection were continued on their regimen, except that efavirenz was discontinued if part of the regimen. During labor, intravenous zidovudine was given as a loading dose of 2 mg/kg over one hour, followed by a continuous infusion of 1 mg/kg per hour until the cord was clamped. The postpartum component consisted of the administration of zidovudine syrup (2 mg/kg orally four times daily) to the infant for the first six weeks of life, beginning 8 to 12 hours after birth for the prevention of MTCT. Elective cesarean delivery was recommended until 2003 after which, vaginal delivery became the standard in mothers with viral load below 1000 copies/mL, unless the patient opted for cesarean delivery.

RESULTS

Thirty-one women with 40 pregnancies were identified as fitting the inclusion criteria for the study. One pregnancy aborted at 13 weeks gestation, the patient had a viral load of 3607 at that time and she was treatment-naïve. The reason for the abortion was unclear. The mode of delivery was elective cesarean delivery in 28 pregnancies (70%) at 36 weeks gestation; 11 (27.5%) had normal spontaneous vaginal delivery. The median CD4+ T-lymphocyte count at about the time of delivery was 536 cells/mm^3 (mean 574, range 183-1142 cells/mm^3), and the median viral load at about delivery time for 25 pregnancies was 1646 copies/mL (73-59071), with only 9 (22.5%) pregnancies having viral loads of more than 1000 copies/mL. Fourteen pregnancies (35%) were receiving highly active antiretroviral therapy (HAART), had undetectable HIV prior to delivery. All were on antiretroviral therapy during pregnancy and delivery. All the newborns were tested at the end of 18 months and tested negative for the HIV-1 screening and PCR assay. Breast feeding was strictly prohibited and complied with by the patients in the study.

DISCUSSION

In a study looking at horizontal versus vertical transmission in 19 HIV-infected people and their contacts, Al Nozha et al found that all nine children born to HIV-infected mothers were HIV-infected.[12] None of the mothers were aware of their serostatus, and none had received antiretroviral therapy, or avoided breast feeding. Our data in this study demonstrate reassuringly successful prevention methods for MTCT. While looking at the reasons for HIV testing in Saudi Arabia, Alrajhi et al[13] showed that the majority of the women with HIV infection acquired the infection from their husbands. Both the natural instincts for motherhood and social pressures culminated in pregnancies in our Saudi population of HIV-infected women. Counseling for conception and childbirth is an essential component of healthcare provided to HIV-infected women. The majority of the pregnancies in our report were planned and coordinated with care providers to ensure tolerance, adherence, and response to antiretroviral therapy before conception. In our hospital, we follow the Public Health Service Task Force recommendations for the management of pregnant HIV-infected women.[14] Our comprehensive care team for HIV-infected women includes social service workers, HIV counselors, pharmacists, and obstetricians.

The MTCT rate of 0% in our study is probably related to the small number of patients. However, other reports like the AmRo study from Netherlands,[15] also had an MTCT rate of 0% among 143 pregnancies. Use of HAART has been associated with preterm deliveries, preeclampsia, and low birth weight.[15] In our study, we had no cases of preeclampsia or preterm delivery; all cesarean deliveries were done electively around 36 weeks of gestation.

MTCT of HIV-1 is multifactorial and may occur even in women with undetectable viral loads.[16] The risk of vertical transmission can be decreased remarkably with elective cesarean section and zidovudine prophylaxis. The PACTG 076 Trial showed clearly that vertical transmission of HIV-1 could be reduced considerably, irrespective of the maternal viral load, with the use of zidovudine.[7,9] The effectiveness of zidovudine in reducing MTCT has been shown to be consistent. Breastfeeding increases the transmission of HIV-1 to the infant by 5-20%. A study from Saudi Arabia looking at the mode of transmission of HIV-1 infection in children showed that 63.5% acquired the infection vertically; 93% were breast fed for almost two years.[11] The compliance to HAART therapy and abstinence from breastfeeding[17] could have contributed to the good outcome in our cohort.

In conclusion, contrary to previous local experience with MTCT, we believe that diagnosis, counseling, antiretroviral therapy, elective cesarean delivery, early antiretroviral therapy for the newborn, and avoiding breastfeeding have contributed to eliminating MTCT among our HIV-infected, pregnant women.

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original article

Analysis of complications in hepatic right lobe living donors

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BACKGROUND AND OBJECTIVES: Living donor liver transplantation (LDLT) has been expanding to adult recipients by using right lobe grafts. However, the incidence of complications is more frequent than that involving left lobe grafts. Hence, we aimed to analyze postoperative complications in right lobe liver donors as a step to improve the results in the donors.

METHODS: Three hundred and eleven right lobe liver donors were retrospectively reviewed between February 1998 and December 2003.

RESULTS: The ages of the donors ranged from 19 to 64 years (median: 46 years). Their body mass index ranged from 16.6 to 34.3 (median: 22.1). The mean duration of the operation was 6.58 (1.25) hours and blood loss was 289 (254) mL. The estimated median donor residual liver volume was 42.2% (range: 20.6-60.3%) and the median hospital stay was 14.5 days (range: 6-267 days). One donor died of liver failure due to small residual liver volume (26%) and steatohepatitis. One hundred and twenty three complications occurred in 104 donors (33.4%). Donors experienced one or more complications. According to the Clavien classification, grade I complications occurred in 71 of the episodes (57.7%), grade II complications in 9 (7.3%), grade IIIa complications in 39 (31.7%), grade IIIb complications in 3 (2.5%), and grade V complications in 1 (0.8%). Biliary complications were the most common and occurred in 37 donors (12%).

CONCLUSION : Right lobe liver donation is a widely accepted procedure that results in the expansion of the indication for LDLT to adults and large children. However, remnant liver size and anatomical variations in the biliary tree represent important risk factors for postoperative complications.

The shortage of donor organs for liver transplantation has led to interest in alternatives to cadaver liver transplantation. Living donor liver transplantation (LDLT), using the left lateral segment or the left lobe, is an acceptable modality to treat end-stage liver disease, especially in pediatric patients. This procedure provides adequate graft mass with excellent results and better donor safety in pediatric patients.[1] However, small-for-size grafts has remained a significant barrier with high morbidity after the expansion of LDLT to adult patients.[2,3] Recently, right hepatic lobe donation had been advocated to overcome size-mismatch between the left lobe and a large-sized LDLT recipient.[4-5] Right lobe donation usually matches graft size although donor safety remains a major concern. In general, a normal liver has been reported to be able to tolerate right lobectomy well, which results in a residual liver volume (RLV) that is as small as 20 to 40%.[6-8] However, surgery-related morbidity, safety of right-lobe donors, and postoperative donor liver function have to be fully investigated and re-evaluated. As donor safety is the top priority in LDLT, the present study focused on the analysis of postoperative complications in right-lobe donors.

METHODS

Study population

Donor candidates were limited to relatives up to the third degree and spouses who showed a strong voluntary wish to donate parts of their livers. After the first-step of obtaining informed consent, donor candidates were subjected to medical screening consisting of blood chemistry analysis, blood count, check for viral hepatitis, abdominal ultrasonography (US), and other tests for general anesthesia. The final candidate was subjected to liver volumetry by using computed tomography (CT). Magnetic resonance cholangiography was performed in a limited number of donors (n=26) with variations in portal venous anatomy such as trifurcation.

Selection criteria for donors were age between 20 and 60 years, healthy, ABO-compatible, graft size >1.0% of recipient body weight, and residual liver volume (RLV=estimated whole liver volume - actual graft weight)/estimated whole liver volume ×100) >30% of the whole liver. Medical or surgical risk factors disclosed by donors were investigated further, if indicated, and final candidacy was determined by a multidisciplinary team. Exceptions in each factor were accepted due to the scarcity of available donors and the severity of the disease in the recipients.

Whole-liver volume and estimated graft and RLV in donors were calculated preoperatively on radiographic CT. CT of the liver for volume determination was performed as described elsewhere.[9,10] No donor candidate was subjected to angiography for arterial anatomy or endoscopic retrograde cholangiography for biliary anatomy. Steatosis of the liver was evaluated only on US and CT.[11] Hepatic steatosis was detected by CT by measuring the liver-to-spleen (L/S) CT value ratio. Steatosis was suspected when the L/S ratio is ≤1.1.[11,12] Such donors were advised to receive dietary and exercise treatment and to abstain from alcohol before donation and reevaluated later. No liver biopsy was performed preoperatively, as per protocol.

Donor operation

Donor operations were performed as described elsewhere:[13] first, complete mobilization of the right lobe, followed by transection of the short hepatic veins on the right side of the inferior vena cava, except in the case of significantly sized right inferior hepatic vein (5 mm). This was followed by isolation of the right branches of the portal vein, hepatic artery, and hepatic duct. At this stage, intraoperative cholangiography was usually performed through the cystic duct and the right hepatic duct(s) was usually transected before the parenchymal transection was started. Transection of the liver was subsequently performed. The demarcation line for transection was determined by temporary clamping of the right portal pedicle. After graft extraction, it was flushed *ex situ* with a histidine-tryptophan-ketoglutarate (HTK) solution through the right branch(es) of the portal vein, weighed, and preserved in the same solution until the time of graft implantation.

Table 1. Donor demographics.

Sex (M/F)	162/149
Age (years)	19-64 (46)
Body weight (kg)	38.4-107.2 (61.2)
BMI (kg/m^2)	16.6-34.3 (22.1)

Numbers in parentheses are median values; BMI, body mass index; HBcAb, hepatitis B core antibody

Postoperative management

Postoperative complications were recorded during and after the initial hospital stay. The follow-up period for the donors was 11 to 81 (median: 32) months. Clavien classification[14] was used to record surgical complications as follows: grade I, alterations from the ideal postoperative course not requiring specialized pharmacological or surgical treatment; grade II, complications requiring specialized pharmacological treatment, blood transfusion, or total parental nutrition; grade III-a, complications requiring invasive intervention without general anesthesia; grade III-b, requiring general anesthesia; grade IV-a, single organ dysfunction; grade IV-b, multiorgan dysfunction; grade V, death; the suffix "d" indicated disability.

Statistical calculations for mean values and standard deviations were performed using the SPSS software package. The chi-square test was adopted for statistical comparison and $P<.05$ were regarded as being statistically significant.

RESULTS

In the period between February 1998 and December 2003, 311 liver transplantations were performed using the right lobe from living donors in the Department of Transplantation and Immunology, Kyoto University, Kyoto, Japan. There were 284 right lobe grafts without the middle hepatic vein (MHV) and 27 with the MHV. Donor demographics are shown in Table 1. The graft donors consisted of 162 men and 149 women and their age ranged from 19 to 64 (median: 46) years. The body weight of the donors ranged from 38.4 to 107.2 (median: 61.16) kg and the body mass index (BMI) from 16.6 to 34.3 (median 22.1) kg/m^2. The donors consisted of 32 fathers, 48 mothers, 78 spouses (33 husbands and 45 wives), 73 siblings (40 brothers and 33 sisters), 73 children (51 sons and 22 daughters), one uncle, one aunt, three nephews, one cousin, and one mother-in-law of the recipients. Thirty-seven donors were ABO-incompatible with their recipients and these ABO-incompatible grafts were selected because of the absence of other compatible candidates. Significant medical history included hypertension in 21 donors, gastro-duodenal ulcer in eight, cardiac arrhythmia in six, diabetes mellitus in seven, anemia in five, asthma in four, angina pectoris in two, gout in two, hyperlipidemia in two, esophageal hernia in one, acute pancreatitis in one, tuberculosis (treated) in one, pyelonephritis in two, pneumonia in one, leiomyoma and hypermennorrhea in two, Kawasaki's disease in one, meningitis in one, multiple liver cysts and solid hemangioma one donor. Previous abdominal surgeries appendicectomy in 40 donors, gynecological surgery in 20, gasterectomy in 1, cholecystectomy in 1, open abdominal biopsy in 1, lumbar disc hernia in 1, cleft lip in 1, nasal fracture operation in 1, mastectomy for cancer breast in 1, and tonsillectomy in one donor. The donors also included 14 positive for hepatitis B surface antigen and two positive for hepatitis C virus (HCV) antibody (status postinterferon therapy), who were adopted as donors in emergency situations as life-saving procedures for recipients positive for the same hepatitis virus.

Surgery and graft profiles

Surgery and graft profiles are shown in Table 2. No allotransfusion was needed for any of the donors during the operation. Residual liver volume (RLV, %) ranged from 20.6% to 60.2% (median 42%). Alternatively, estimated RLV (percent), which uses estimated graft volume instead of actual graft volume, ranged from 20.7% to 55.8% (median 41.2%). Ten donors had a preoperative estimate RLV of less than 30%.

As the safety limit was tentatively estimated as 30% for the estimated RLV, the actual transection line was intentionally shifted to the right in these ten cases. Consequently, only five of these ten donors had a postoperative estimated-actual RLV<30%. On the other hand, nine other donors had an estimated RLV<30% and had a postoperative estimated-actual RLV>30%. It was found that the rate of postoperative complications was significantly higher in donors with postoperative estimated-actual RLV<30% with P=.001.

Postoperative liver functions

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum total bilirubin (TB) levels returned to normal levels after the end of the first week in 15% of the donors. These levels had returned to normal in 50% and 90% of the donors by the end of the second and third weeks respectively. AST, ALT, and serum total bilirubin had decreased to normal levels within one month after the transplant surgery in all donors. The mean peak levels for AST, ALT, and serum TB were 348.8 (151.7) U/L, 354 (174.9) U/L, and 3.2 (1.4) mg/dL respectively. On the seventh postoperative day, AST, ALT, and TB levels were 69.6 (35.8 U/L), 128 (62.4 U/L), and 1.28 (1.04 mg/dL). The hospital stay was for 14.5 days (range: 6-267 days).

Table 2. Surgical and graft profile.

Surgical time (hr)	3.25-11.15 (6.6)
Blood loss (mL)	10-2300 (282)
Graft weight (g)	415-1270 (670.95)
GRWR (%)	0.55-2.39 (1.16)
Estimated RLV (% total)	20.7-55.82 (41.2)
Residual liver volume (% total)	20.6-60.2 (42.0)
Macrovesicual steatosis (mild to moderate)	20.0%

Numbers in parentheses are median values; GRWR, graft-to-recipient weight ratio; RLV, residual liver volume

Postoperative complications

All (but one) donors are alive and healthy along the follow up period (which extends to 7 years), although 30% of the donors experienced one or more complications. One donor died of liver failure due to a small remnant liver (26%) and concomitant nonalcoholic steatohepatitis which was diagnosed postoperatively in spite of a rescue domino transplant. The preoperative ultrasound and CT showed mild to moderate steatosis and volumetry showed the residual volume to be 30%. The steatohepatitis was diagnosed by liver biopsy taken during the operation. After the operation, the donor developed continuous progressive liver failure which ended with a domino liver transplantation. After initial improvement, the condition deteriorated again until death due to multiple organ failure and sepsis after 267 days of hospital stay. As any complication in a healthy donor is significant, both minor and major complications were recorded and consequently, a total of 123 complications were recorded to occur in 104 donors (Table 3).

Biliary complications were the most common complications and constituted 40/122 episodes (32.7%) in 37/311 donors (about 12 % of all donors); 34/40 bile leakages (85% of biliary complications) and 6/40 biliary strictures (15% of biliary complications) (three of which followed biliary leakage). Biliary leakage was di-

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Table 3. Postoperative complications.

	Number of episodes	Clavien classification				
		GI	GII	GIIIa	GIIIb	GV
Biliary complications						
Bile leakage	34	8		26		
Biliary stricture	$6^{a,b}$			5	1	
Other abdominal complications						
Persistent fluid collection	38	38				
Skin wound infection	11	11				
Massive ascites (>500 mL/day)	4			4		
Small bowel obstruction	5^{b}		4		1	
Hyperamylasemia (>300 IU/L)	3	3				
Portal vein thrombosis	1b				1	
Drug induced hepatotoxicity	1		1			
Diverticulitis (ascending colon)	1		1			
Allergic reaction	1	1				
Extra-abdominal complications						
Pleural effusion	4			4		
Pulmonary embolism (including suspected cases)	3		3			
Fever of unknown origin	8	8				
Angina pectoris	1	1				
Radial nerve palsy (temporary)	1	1				
Death	1					1
Total	**123**	**71**	**9**	**39**	**3**	**1**

[a] Three episodes followed bile leak; [b] One each case required relaparotomy

agnosed with a laboratory test for bilirubin in the drain in 22 cases, hepatobiliary imino-diacetic acid (HIDA) scan in ten cases, and endoscopic retrograde cholangiopancreatography (ERCP) in five cases. The cause of the leakage was the cut surface of the liver in 30 donors (81%) and the site of cut of the bile duct in three donors (8%) but not defined in four donors (11%). All biliary leakages were managed conservatively, either by drainage through the drainage tube until spontaneous disappearance in eight cases, or additional percautaneous drainage after the removal of the drain tube in 15 cases, or the receipt of endoscopic nasobiliary drainage after percautaneous drainage in 12 cases. Bile leakage occurred between one and 41 (median 13) days after donation. Six biliary strictures occurred, three of them followed bile leakage. They were diagnosed on the basis of elevated total serum bilirubin levels and by ERCP. One patient required conversion to hepaticojejunostomy 20 months after donation. The remaining cases were managed successfully with temporary endoscopic retrograde biliary balloon dilatation and stenting. During the period of follow-up, the stent was changed after six months in four donors with biliary strictures and was removed from the other two donors without any complication. Cholestasis occurred in 17 donors (5%) when the total bilirubin levels reached >5 mg/dL. These donors improved upon the administration of urthodesoxycholic acid medication. Biliary ducts were one in 249 donors (80%), two ducts in 59 donors (19%), and three ducts in three donors (1%). The biliary complications due to bile duct leakages (not in the cut surface) were significantly more in the donors with more than one bile duct (P=.0001).

Other abdominal complications include persistent asymptomatic fluid collection diagnosed by ultrasonography without any symptoms in 38 donors (12%). Fluid collection was not related to any known risk factors for donors such as steatosis, medical problems, or residual volume. One of the five cases had small bowel obstruction and required surgical adhesiolysis ten months after donation.

Portal vein thrombosis occurred in one patient which was diagnosed on day 7 by the sudden appearance of ascites along with a loss of the portal flow signal on Doppler US. Emergency laparotomy revealed a kink in the reconstructed portal vein. This area was dissected from the surrounding tissue and the kink was corrected. No further problems were encountered with portal vein flow in the follow-up period.

Pleural effusion necessitating drainage occurred in four cases and pulmonary embolism was suspected in three cases with mild hypoxemia and perfusion defects in pulmonary scintigrams. All the three cases recovered with the use of heparin and urokinase.

According to Clavien classification,[14] there were 123 complications which occurred in 104 donors (33.4 %). Grade I complications occurred in 71 (57.7%), grade II complications in 9 (7.3%), grade IIIa in 39 (31.7%), grade IIIb in 3 (2.5%), and grade V complication in 1 patient (0.8%). The rate of rehospitalization was about 16% in all donors according to various indications (Clavien grades II, III, and V). The incidence of reoperation was 0.9% in all donors (Clavien grade IIIb).

DISCUSSION

Donor safety is the key issue for the wider application of LDLT. No effort should be spared in avoiding complications by appropriate patient selection, controlling blood loss, meticulous surgical technique, and postoperative care. This would minimize complications and ensure early return to the normal activities of life. LDLT was first applied to pediatric patients using the left lateral segment or left-lobe graft for donor safety. However, after experiencing liver failure in small-sized grafts in adults and large children, it was concluded that the risk-to-benefit ratio would favor the use of right-lobe graft in LDLT.[15]

Right lobectomy from healthy donors was introduced to the Department of Transplantation Immunology, Kyoto University, Kyoto, Japan in February 1998. This has enabled us to minimize postoperative failure in adult patients.[16] Analysis of postoperative complications in these donors is important because any complication could lead to death.

Since the initiation of the program of right-lobe donation until the end of December 2003, 311 hepatic right liver transplants were performed. Postoperative analysis of complications revealed the occurrence of 123 complications in 104 donors (33.4% of donors). The incidence and the severity of complications were found to decrease by acquiring more experience. This was true with regard to technical problems (vascular and biliary). One donor developed portal vein thrombosis which occurred early in the protocol. Biliary problems occurred in 12% of the donors, which also decreased by the surgical teams' gaining experience in the technique of harvesting. Biliary complications had occurred in 22 of the first set of 155 donors (14%) and in 15 of the second set of 156 donors (9.5%). This improvement indicates the importance of the learning curve.

The incidence and variety of complications in living donors vary with the program and with the donor population. The differences are also affected by the lack of uniformity in assessing and defining complications. Published reports on donor outcomes indicated a wide range of complication rates that varied between 9 and 67%.[4,17-23] This may be attributed to the change in the definition of each complication.

In the present study, biliary complications constituted 40 episodes in 37 donors (about 12%). Bile leakage including minor and self limiting leakage occurred in 34 donors (about 11%). The cause of the leakage was the cut surface of the liver in 30 donors (81%) and from the bile duct in three donors (8%) but not defined in four donors (11%). Biliary stricture occurred in six donors (about 2%); three of the six cases (2%) of biliary strictures followed bile leakage. One donor with stricture required conversion to Roux-en-Y hepaticojejunostomy; cholestasis occurred in 17 donors (5%). Persistent fluid collection is the second most frequent complication and constituted 38 episodes in 38 donors. The rate of rehospitalization was about 16% for all donors according to various indications (Clavien grades II, III, and V). The incidence of reoperation was 0.9% for all donors (Clavien grade III-b).

The incidence of biliary leakage in our series was comparable with that in other studies.[24] Cases of biliary leakage from the bile duct were followed by stricture which was managed successfully with ERCP. Although great care was adopted in harvesting the donor graft and in cutting the ducts in the donor side with the use of cavitational ultrasonic surgical aspirator (CUSA) and clipping and tying the small bile ducts on the cut surface, the resolution of biliary complications was still slow. This may be due to the selection of donors with more than one bile duct or with complex biliary anatomy as the only available donors for critically ill recipients. Thus, our learning experience must have clearly decreased the incidence of biliary complications although the improvement was not statistically apparent due to the increase in acceptance of donors with complex biliary anatomy. In other series, approximately 40% of right-lobe donors experienced at least one complication during the year after the transplant surgery.[24] Biliary leakage or stricture complicated the postoperative course in 6% of the donors,[25] an observation confirmed by previous reports.[26,27] Rehospitalization occurred in 8.5% of 75 donors, 5% of whom required reoperation.[25] A recent multicenter survey in five Asian liver transplantation centers reported complications and long-term outcomes in 1,058 live donors: more than half of these were right-lobe donors (561 of 1,058 donors).[28] Although the overall morbidity rate was 15.8%, right-lobe donors had a greater incidence of complications (28%) and more serious complications compared with left-lateral segment (9%) and left-lobe donors (7.5%) respectively. The most common serious problem encountered in patients undergoing right-lobe donation was biliary complications, in particular, cholestasis, (7%), bile leakage (6%), and biliary stricture (1%). Other serious complications included portal vein thrombosis (0.5%), intra-abdominal bleeding (0.5%), and pulmonary embolus (0.5%). Reoperation was necessary in 17 donors (1.1%) for small bowel obstruction, biliary stricture, bile leakage, bleeding, portal vein thrombosis, postoperative ileus, and incisional hernia.

Only 15% of patients were followed up for longer than three months; however, six donors had residual morbidity, including five patients with biliary complications and one patient with chronic renal failure secondary to intravenous contrast. One donor experienced sudden death while exercising three years after the transplant.[28]

A review of the experience in the USA with adult LDLT reported a 4% biliary complication rate in donors who required surgical intervention, endoscopic retrograde cholangiopancreatography, or percautaneous catheter drainage of a postoperative biloma.[29] Surgical re-exploration was required in two patients for portal vein thrombosis and small-bowel obstruction. Additional complications reported in the donor population include neuropraxia, phlebitis, pressure sores, pleural effusion, pneumonia, pulmonary embolus, deep venous thrombosis, prolonged ilieus, and incisional hernia.[29]

Beavers et al. reviewed the incidence of donor morbidity associated with right lobectomy in LDLT between 1995 and 2001.[30] Reported complications in right-lobe donors ranged from 0 to 67% with a crude complication rate calculated to be 31% (54 events in a 74 donors). The wide variation in complication rates among centers reflects the inconsistency in defining what constitutes a complication, with some centers reporting all adverse events and others reporting only major or life-threatening complications. On an average, right-lobe donors spent 9.9 days in the hospital, returned to work 2.4 months postoperatively, and felt completely recovered by 3.4 months.[30]

There was an acceptably good correlation between estimated graft volume and actual graft weight, as reported previously.[10] However, a significant error was observed in some cases, which may have contributed to smaller estimated RLV than the actual one, or may have been attributable to an intentional shift of the transaction plane to the left side on the basis of sufficient size of the estimated RLV.

One donor died of liver failure due to a small remnant liver (26%) and concomitant nonalcoholic steatohepatitis that was diagnosed postoperatively, in spite of rescue domino transplant. This is the first donor death to be reported from Asia; the donor was a woman who donated her liver to her teenage daughter.[31] The preoperative assessment showed mild to moderate steatosis and the residual volume was estimated to be 30%. The actual volume after the operation was 26% which may have been due to the shift of the resection margin to the left. The lesson learned from this case was the need to avoid hesitation in taking liver biopsy from donors, especially when the estimated residual volume was ≤30%, so that no additional risk factors, such as steatohepatitis, were imposed on the remaining part of the liver A total of nine deaths occurred worldwide (two deaths in left lateral segmentectomy donors and seven deaths in right lobectomy donors). Three additional donors have undergone liver transplantation because of complications related to right-lobe donation.[28,31,32] Unlike the two deaths of left lateral segmentectomy donors (pulmonary embolus and anaphylaxis), deaths occurring in right-lobe living donors have resulted from multiple organ failure and sepsis. Although donor deaths have resulted from either technical failures or problems in the early postoperative period, these cases underscore the reality that living donation is associated with a small, but real, possibility of mortality. The increasing use of living-related donation should not delay the seeking of ways to obviate the need for transplantation. Also, the optimal graft is a whole liver from a cadaveric donor. Our efforts must go into making full use of the cadaveric pool. We must know that only a proportion of candidates will be helped by living liver donors either due to scanty or due to medical contraindications in living donors.

Finally, the morbidity associated with right-lobe donation is significant and probably occurs in a third of all the donors. However, close attention and meticulous techniques must be used to prevent surgical complications such as bile leakage and cholestasis. Vigilant examination is required to avoid inappropriate donor selection. Liver biopsy can be resorted to in donors with a suspicious steatosis.

In the present study, donors were continuously followed up for any complication. The precise rate of incidence of late complications in right-lobe donors is speculative because few, if any, studies exist reporting the follow-up of donors beyond the first postoperative year. Fortunately, all the donors' liver function test results normalize by the end of the first month after liver donation and before regeneration is complete.

In conclusion, LDLT using right lobe grafts for larger patients is another step that can increase donor risk. Donor safety has been and must continue to be the primary measure of success of this technique. With technical refinements backed with precise knowledge on anatomical variations and physiology, we believe right lobe LDLT has become a very effective treatment modality with acceptable risk to both donors and recipients.

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original article

Prevalence of elevated hepatic transaminases among Jordanian patients with type 2 diabetes mellitus

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BACKGROUND AND OBJECTIVES: Since the extent of elevation of liver transaminases in type 2 diabetics in Jordan and most of the Middle East is unknown, we estimated the prevalence of elevated liver transaminase levels among patients with type 2 diabetes and determined associated risk factors.

METHODS: This study was performed on 1014 consecutive type 2 diabetic outpatients who attended the National Center for Diabetes, Endocrinology and Genetics in Amman, Jordan. The patients' age ranged between 26-85 years with a mean age of 56.8 (+9.8). Three- hundred and fifty three (54.5%) were males with a median age of 58 years (ranging between 26-82 years), and four hundred and sixty one (45.5%) were females with a median age of 57 years (ranging between 28-85 years). Body mass index, waist circumference, lipid profile, and hepatic transaminase levels were recorded. Ultraonography was performed in those with elevated alanine transaminase levels.

RESULTS: Overall, the prevalence of elevated alanine transaminase (ALT) level was 10.4% (n=105) with the gender-wise prevalence being 12.8% (n=71) in men and 7.4% (n=34) in women. The prevalence of elevated aspartate transaminase (AST) levels was 5.4% (n=56) with the gender-wise prevalence being 5.6% (n=31) in men and 5.4% (n=25) in women.. Only 4.5% (n=44) showed elevated levels of both ALT and AST. Male gender (OR=2.35, CI:1.5-3.8) and high waist circumference (OR=1.9, CI:1.2-3.2) were associated with increased risk of elevated ALT levels. Younger patients had a higher tendency to have elevated ALT compared to those over 65 years (OR=12.4 for patients aged 25-45years, and OR=5.8 for those who were 45-65 years old). Non-insulin use was associated with a high odds ratio for elevated ALT levels (OR=1.7, CI: 1.1-2.9).

CONCLUSIONS: Elevated ALT and AST levels are found in 10.4% and 5.4% of our type 2 diabetic patients respectively. Male gender, younger age, higher waist circumference; as an indicator of central obesity, as well as non insulin use are independent predictors of elevated liver transaminase levels.

Diabetes mellitus is known to be associated with a number of liver disorders, including isolated elevation of liver enzyme levels, nonalcoholic fatty liver disease (NAFLD), and other chronic liver disorders like hepatitis C infection (HCV), cirrhosis and hepatocellular carcinoma.[1-3] NAFLD is a common indolently progressive liver disease and may range from simple steatosis of the liver, through the more severe nonalcoholic steatohepatitis (NASH) to advanced fibrosis and cirrhosis.[4] NAFLD resembles alcohol induced liver disease, but develops in subjects who are not heavy alcohol drinkers, and who have negative tests for viral, autoimmune and metabolic liver disease. NAFLD has been related to insulin resistance and obesity, suggesting that it may represent yet another component of the metabolic syndrome.[5,6]

It is thought that decreased insulin sensitivity, a cornerstone in the pathogenesis of type 2 diabetes (T2D), activates lipolysis leading to increased plasma levels of non-esterified fatty acids. A resultant chronic increase in fatty acid flux from the fat stores (mainly abdominal) to non-adipose tissues such as the liver, contributes to the hepatic steatosis.[7,8] Abnormal liver function test results are more common in diabetes mellitus than in the non diabetic population as well as in patients with T2D than in those with type 1diabetes.[9,10]

Elevated activities of the two serum transaminases; alanine transaminase (ALT) and aspartate transaminase (AST) maybe associated with liver disease. Elevation of the levels of any of the two enzymes has been found in 7.9% of the general population,[11] whereas the prevalence of high ALT levels may reach 20% in diabetics[12]. Elevation of these enzymes is strongly related to obesity, diabetes and dyslipidemia, and their measurement may act as a surrogate marker of NAFLD presence.[2,4] Of the two enzymes, ALT appears to have a role in gluconeogenesis,[13] and seems to be more related to liver fat accumulation than AST.[14] Some authors have suggested that minor elevation of this enzyme's level may be a good predictor of mortality from liver disease.[15] A study of the association of serum ALT activity and ten-years' risk of cardiovascular disease (CVD)in participants of the Third National Health and Nutrition Examination Survey (NHANES- III) had reported that those with elevated ALT levels had a higher calculated CVD risk than those with normal ALT activity, if viral hepatitis or excessive alcohol consumption were excluded.[16]

The activity of ALT in the hepatocytes is 7000 fold greater than in the serum,[17] and this abundance is the reason for using it as a marker for NAFLD in many epidemiological studies.[18] Clark et al. proposed that elevated AST or ALT levels are predictive of the presence of NAFLD if two basic criteria are met: 1) exclusion of alternative chronic liver diseases, e.g. alcoholic liver disease, hepatitis B or C infection, and hemochromatosis; and 2) presence of features of the metabolic syndrome.

The magnitude of elevated levels of liver transaminases in the Jordanian population is currently unknown, and few studies in the Middle East have addressed this issue.[19-21] This study was conducted to estimate the prevalence of elevated liver transaminase levels among patients with type 2 diabetes and to determine the associated factors.

METHODS

All patients aged ≥25 with type 2 diabetes who attended the National Center for Diabetes, Endocrinology and Genetics (NCDEG) Amman, Jordan in the period between August-December 2007 were consecutively invited to participate in the study. A total of 1014 patients agreed to participate with a response rate of 98.4%. Selecting consecutive patients over a period of five months in the year and high response rate make the sample more representative of the population of patients attending NCDEG. The sample size of 1014 patients exceeded the minimum required sample of 682 that was calculated at a level of significance of 0.05 and power of 80% assuming that the expected prevalence is 10% and null hypothesis value is 7%. The sample size was calculated using EpiCalc (version 1.02).

Patients were interviewed during their periodic visit to the center, and their data were recorded in a standardized data sheet. Data collected included age, gender, marital status, level of education and co-morbid conditions like hypertension and dyslipidemia. Patients were asked for history of alcohol consumption and the medications used, mainly hepatotoxic drugs as steroids, antiepileptics, amiodarone and antineoplastic drugs. The study was approved by the Ethics Committee in NCDEG and informed consent obtained from all participants.

Anthropometric measurements

Blood pressure readings were taken with patients in the sitting position. Patients were labeled as hypertensive if they were already on antihypertensive medication, or were found to have systolic blood pressure equal or higher than130 mmHg and/or diastolic blood pressure equal or higher than 80 mmHg on two occasions separated by at least one week.[22] Body weight was taken while the patients barefooted and in light clothing, using a Detecto® scale with accuracy of ±100 g. Standing height was measured without shoes to the nearest cm using a stadiometer with the shoulders in a relaxed position and the arms hanging freely Body mass index. (BMI) (kg/m^2) was calculated as the ratio of weight (kilograms) to the square of height (meters). Patients' BMI was classified according to WHO classification,[23] as being normal (BMI; 18.5 to 24.9 kg/m^2), overweight (BMI; 25 to 29.9 kg/m^2) or obese (BMI>30 kg/m^2). Waist circumference was measured in a standing position at the level of the umbilicus, using non stretchable tailor measuring tape and results were analyzed as categorical variable with the circumference considered abnormal if it exceeded 88cm in women, and 102cm in men.[24]

Biochemical tests

Morning samples of venous blood were collected from patients after fasting for at least 14 hours and tested for glucose, hemoglobin A1C (HbA1c) [Using High Performance Liquid Chromatography method, supplied by Bio-Rad], for the liver enzymes; ALT and AST [Using COBAS INTEGRA systems to measure the quantitative determination of the catalystic enzyme activity, supplied by Roche Diagnostics], and for lipid profile including total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG), and low density lipoprotein (LDL) [By using the Enzymatic Colometric method by COBAS INTEGRA systems supplied by Roche Diagnostics]. Elevated ALT and AST levels were defined as enzyme activity >40 U/L, and >38 U/L respectively according to the clinical assay adopted by the center's laboratory. The degree of ALT elevation was categorized to mild (one to two times the upper limit of normal), moderate (>two to three times the upper limit of normal) and marked (more than three times the normal). To overcome the day to day variation, liver enzyme measurements were repeated after one month in patients who showed high transaminase values.[25] As all the patients visited the clinic regularly, repeated serum transaminases measurements were available for each at least two times per year. Of those patients with normal ALT levels, none were found to have had any previous increases in the enzyme levels. The periodic liver function tests performed for all the patients in the Center also helped to detect and monitor any future changes that might be related to the use of drugs which are potentially hepatotoxic like statins.

Serological tests for the hepatitis B surface antigen (HBsAg) and HCV were requested for patients who were not initially known to be seropositive for viral hepatitis. Viral hepatitis screen was done only for patients who showed elevation in transaminase readings.HBsAg and HCV were measured using micro particle enzyme immunoassay (MEIA) technology.

Radiological examination

NAFLD was diagnosed by an ultrasound scan of the liver performed by an experienced radiologist who was blinded to the laboratory values, by using Philips HDI 5000 Sono CT with a probe array (C5-2). A fatty liver was diagnosed if there was diffuse increase in liver echogenicity as compared with that of the kidneys.[26] Only patients with abnormally high ALT values had undergone liver scans, after excluding causes that might elevate the transaminase levels.

Criteria for exclusion

Subjects who were excluded from the study, were those who reported alcohol consumption, patients who are known to be seropositive for HBsAg or HCV antibodies, and those who had a clinical or biochemical evidence of autoimmune hepatitis, primary liver cirrhosis, hemochromatosis or Wilson disease.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS), version 11.5. Continuous variables were summarized as means (SD), and categorical variables as frequencies and percentages. The chi-square (χ^2) test was used to determine the association of elevated ALT and AST levels with different variables. Multivariate analysis using logistic regression analysis was used to determine factors associated with elevated ALT and AST levels. The association of a particular variable was expressed as odds ratio (OR) with a 95% confidence interval (CI). A two-tailed *P* value of .05 was considered statistically significant.

RESULTS

The demographic, clinical and anthropometric characteristics of the participants have been displayed in Table 1. Among the 1014 patients enrolled in this study, 54.5% (n=553) were males and 45.5 % (n=461) were females. The mean age was 56.8+9.8 years. Seven hundred and thirteen patients (70.3%) were between the ages of 45 and 65 years and 445 (44.9%) patients had their diabetes diagnosed during the last five years. Three hundred and fifty patients (34.5%) were overweight and 572 (56.4%) were obese.

Insulin was mostly prescribed for the old patients than for the younger group ($P<.005$). Hypertension was found to be most prevalent in patients who were over the age of 65 years ($P<.005$).

Prevalence of elevated transaminases

Overall, the prevalence of elevated alanine transaminase (ALT) was 10.4%(n=105) with the gender-wise prevalence of 12.8% (n=71)(95% Confidence Interval (CI): 10.1, 15.5) in men, and 7.4% (n=34) (95% CI: 5.1, 9.7) in women (Table 2). The prevalence of elevated AST was 5.4% (n=56) (95% CI 4.1, 6.9) with the gender-wise prevalence of 5.6 %(n=31) (95%CI: 3.7, 7.5) in men and 5.4 % (n=25) (95% CI: 3.4, 7.5) in women). Only 4.5% (n=44) showed elevated levels of both ALT and AST. Of patients with high ALT levels, 88 patients (83.8%)

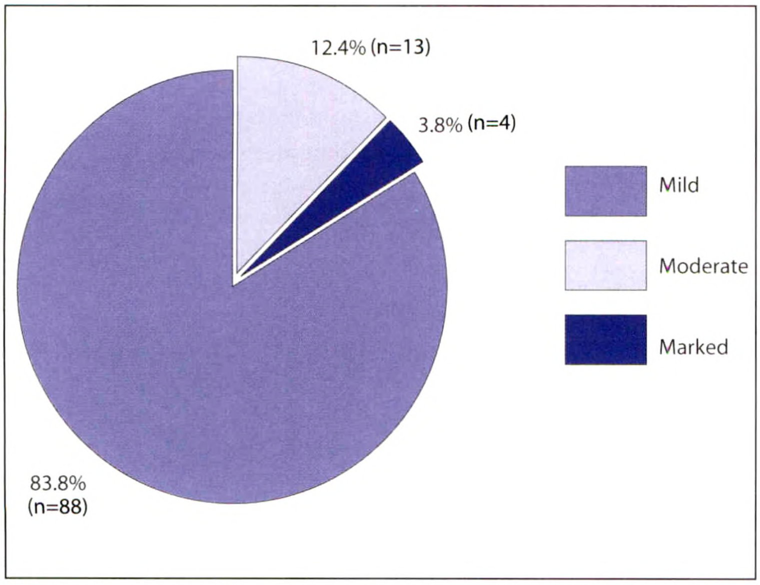

Figure 1. Degree of elevation of ALT enzyme level in patients with high readings. Mild, moderate and marked elevation indicate ALT levels 2 times, 2-3 times and more than 3 times the upper limit of normal value respectively.

Table 1. The demographic, clinical and anthropometric characteristics of the 1014 patients with type 2 diabetes.

Variable	n (%)
Gender	
Male	553 (54.53)
Female	461 (45.46)
Age (years)	
25-45	146 (14.39)
46-65	713 (70.31)
> 65	155 (15.28)
Marital status	
Single	11 (1.1)
Married	974 (96.1)
Divorced	5 (0.5)
Widow	24 (2.4)
Body mass index (kg/m^2)	
18.5-24.9	92 (9.07)
25-29.9	350 (34.51)
≥30	572 (56.41)
Diabetes duration (years)	
<5	455 (44.87)
5-10	271 (26.72)
11-15	125 (12.32)
>15	163 (16.07)
Waist circumference (cm)	
>102 (males)	282 (50.99)
>88 (females)	433 (93.92)
HBA$_{1c}$ (%)	
>7	676 (66.66)
Lipids (mg/dL)	
LDL>100	498 (49.11)
TG>150	394 (38.85)
HDL<40 (males)	277 (50.09)
HDL<50 (females)	279 (60.52)
Liver enzymes U/L	
ALT>40	105 (10.35)
AST>38	55 (5.42)
ALT>40 and AST>38	46 (4.53)
Statin use	
Yes	642 (63.3)
No	372 (36.6)

Values are numbers of patients and percentage; total n=1014

had mild, 13 (12.4%) had moderate, and only four patients (3.8%) had marked elevation of the enzyme activity (Figure 1). The prevalence of elevated ALT and AST levels according to demographic, clinical, and anthropometric characteristics is shown in Table 2. Male gender (OR=2.35, CI: 1.5-3.8) and high waist circumference (OR=1.9, CI: 1.2-3.2) were associated with an increased risk of elevated ALT levels. Younger patients had a higher tendency to have elevated ALT compared to those over 65 years (OR=12.4 for patients aged 25-45 years and OR=5.8 for those who were 45-65 years old). Non-insulin use was associated with a high odds ratio for elevated ALT levels (OR=1.7, CI: 1.1-2.9). High ALT levels were significantly associated with the duration of diabetes: 14.3% (CI 11.2, 17.4), 9.2% (CI 5.9, 12.6), 8.0% (CI 3.4, 12.6), and 3.7% (CI 0.9, 6.4) in patients with diabetes dating to <5, 5-10, 11-15 and >15 years respectively. The prevalence of elevated AST level decreased with increased age and increased duration of diabetes (Table 2).

Multivariate analysis of risk factors for elevated ALT and AST

The only factors associated with elevated ALT levels in multivariate analysis were gender, waist circumference, age, and non- insulin use (Table 3). Male gender (OR=2.35, CI: 1.5-3.8) and higher waist circumference (OR=1.9, CI:1.2-3.2) were associated with increased prevalence of elevated ALT levels. Younger patients had a higher tendency to have elevated ALT levels compared to those over 65 years (OR=12.4 for patients between 25 and 45 years and OR=5.8 for those between 45 and 65 years). Non-insulin use was associated with high odds ratio for elevated ALT levels (OR=1.7, CI: 1.1-2.9). For elevated AST levels, only the age of 25-45 years was significantly as-

Table 2. Prevalence of elevated alanine transaminase and aspartate transaminase levels according to demographic, anthropometric and relevant characteristics.

Variable	Total	High ALT (%)				High AST (%)			
		n	Prevalence (95% CI)	χ^2	*P* value	n	Prevalence (95% CI)	χ^2	*P* value
Gender									
Male	553	71	12.8 (10.1, 15.5)	8.1	0.004	31	5.6 (3.7, 7.5)	0.02	.899
Female	461	34	7.4 (5.1, 9.7)			25	5.4 (3.4, 7.5)		
Age groups (years)									
25-45	146	29	19.9 (13.6, 26.1)	29.6	<0.005	12	8.2 (3.8, 12.7)	7.0	.031
46-65	713	73	10.2 (8.1, 12.4)			40	5.5 (3.9, 7.3)		
>65	155	3	1.9 (0.0, 4.0)			4	2.6 (0.1, 5.1)		
Smoking status									
Current	135	13	9.6 (4.8, 14.5)	0.11	0.947	8	5.9 (1.9, 9.9)	0.33	.849
Non	806	84	10.4 (8.4, 12.5)			45	5.6 (4.0, 7.2)		
Past	73	8	11.0 (3.8, 18.1)			3	4.1 (0.0, 8.7)		
Diabetes duration (year)									
<5	455	65	14.3 (11.2, 17.4)	15.7	0.001	37	8.1 (5.6, 10.6)	10.9	.012
6-10	271	25	9.2 (5.9, 12.6)			10	3.7 (1.4, 5.9)		
11-15	125	10	8.0 (3.4, 12.6)			4	3.2 (0.1, 6.3)		
>15	164	6	3.7 (0.9, 6.4)			5	3.1 (0.4, 5.7)		
Body Mass Index (kg/m^2)									
18.5-24.9	92	9	9.8 (3.9, 15.7)	0.62	0.732	4	4.3 (0.2, 8.5)	0.93	.628
25-29.9	350	33	9.4 (6.5, 12.4)			17	4.9 (2.6, 7.1)		
>30	572	63	11.0 (8.5, 13.5)			35	6.1 (4.2, 8.1)		
Waist circumference(cm)									
Normal	299	27	9.0 (5.9, 12.2)	0.14	0.371	12	4.0 (1.8, 6.2)	1.4	.226
High	715	78	10.9 (8.7, 13.1)			44	6.2 (4.4, 7.9)		
Hemoglobin A1C (%)									
<7	338	34	10.1 (7.0, 13.2)	0.05	0.827	19	5.6 (3.2, 8.1)	0.01	.923
>7	676	71	10.5 (8.3, 12.7)			37	5.5 (3.8, 7.2)		
LDL (mg/dL)									
<100	516	48	9.3 (6.9, 11.7)	1.2	0.263	22	4.3 (2.5, 6.0)	3.2	.074
>100	498	57	11.4 (8.7, 14.2)			34	6.8 (4.6, 9.0)		
HDL (mg/dL)									
Normal	463	38	8.2 (5.8, 10.6)	1.4	0.040	20	4.3 (2.5, 6.2)	1.96	.162
Low	551	6	12.2 (9.5, 14.8)			36	6.5 (4.5, 8.6)		
Triglycerides (mg/dL)									
<150	620	60	9.7 (7.4, 11.9)	0.79	0.374	32	5.2 (3.4, 6.9)	0.40	.527
>150	394	4	11.4 (8.4, 14.5)			24	6.1 (3.7, 8.5)		
Hypertension									
No	239	35	14.6 (10.16, 19.13)	6.2	0.013	15	6.3 (3.2, 9.4)	0.33	.563
Yes	774	70	9.0 (7.02, 11.06)			41	5.3 (3.7, 6.9)		
Insulin use									
Yes	711	21	3.0 (1.7, 4.2)	5.4	0.019	14	4.6 (2.3, 7.0)	0.674	.412
No	303	84	27.7 (22.8, 32.6)			42	5.9 (4.2, 7.6)		

CI, Confidence interval, χ^2, Chi-square test

Table 3. Multivariate analysis of factors associated with elevated ALT and AST*.

Variable	Odds ratio (95% CI)	*P* value
Gender		
Female	1	<.005
Male	2.4 (1.5-3.8)	
Age group (years)		
>65	1	
46-65	5.8 (1.8-18.7)	<.005
25-45	12.4 (3.7-41.9)	.003
Waist circumference (cm)		
Normal**	1	.008
High	1.9 (1.2-3.3)	
Insulin use		
Yes	1	.030
No	1.7 (1.1-2.9)	

*The only variable associated with elevated AST was age (OR=2.4, 95% CI: 1.05, 11.0) (25-45 year compared to >65 year); **Normal values for men <102 cm, and <88cm for women.

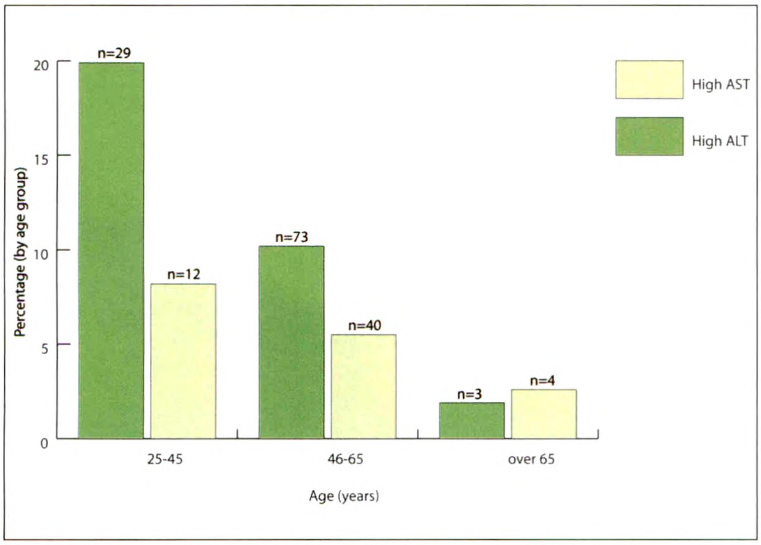

Figure 2. Pattern of ALT and AST elevation in the study population, according to different age groups. Note the higher prevalence of enzyme activity in the younger group with a decline as patients advance in age, and the relatively higher AST in the older group presumed to be a consequence of increased fibrosis.

sociated with higher risk of elevated enzyme activity compared to age of more than 65 years.

DISCUSSION

The prevalence of elevated transaminase levels is not known in the Jordanian population. In earlier studies, applying different methodology and enrolling variable population sample sizes as well as considering different cut-off values for ALT readings has yielded variable prevalence rates.[9,10,12,19,27] The present study shows that ALT was elevated in 10.4% of our T2D patients (12.8% in males and 7.4% in females). Our results are consistent with the finding of Erbey et al. who reported prevalence rates of 10.7% and 5.3% in type 2 diabetic men and women respectively.[10] Furthermore, West et al. reported a higher rate of 12.1%; 14.4% in men and 9.3% in women with T2D.[27]

This study showed that younger diabetic patients were more likely to have high ALT values than the older patients. However the older patients showed elevated AST activity (Figure 2). Supported by earlier studies,[2,7,28] this finding suggested that severe steatosis denoted by a higher release of the ALT enzyme in response to hepatocytes derangement, tends to occur earlier in the disease process. As a marker of hepatocyte integrity the ALT activity decreases as steatosis progresses whereas inversely a rise in the AST level has been noticed in the older patients. The latter observation can be attributed to the fact that the clearance of this enzyme is mainly accomplished by the liver sinusoidal cells. While there is no effect from the necroinflammatory activity on AST level, advancing fibrosis which injures the sinusoidal cells leads to the relative increase in serum AST.[29]

The study concluded in multivariate analysis that both male gender and high waist circumference is independent predictor of ALT levels. We assume that the higher abdominal fat content in men compared to women would explains this finding which has been previously reported in other studies [27,30,31]

Body mass index was not associated significantly with elevated ALT levels. This finding can be attributed to the fact that elevated ALT levels are more related to central fat distribution than to general obesity. The most obvious explanation for this is that in the presence of insulin resistance, the larger mass of adipose tissue in abdominally obese subjects causes an inappropriate suppression of lipolysis and increased flux of non-esterified free fatty acid from visceral fat to liver. Hence, there is an increase in the size of hepatic FFA pool which favors the accumulation of triglycerides in the hepatocytes. The triglyceride overload provides abundant amounts of substrate for non-oxidative pathways, in addition to causing mitochondrial dysfunction. These lipid-induced changes are believed to ultimately lead to cell apoptosis.[32,33]

The findings of our study are similar to those of a study conducted by West et al, showing that insulin use may be protective against the effects of elevated ALT levels. Although this finding suggests that administration of insulin to diabetic patients may help to avoid harmful involvement of the liver by inflammation and fibrosis, it requires further investigation. This is because earlier studies have reported that the standardized mortality ratio from cirrhosis in diabetics was higher in patients who received insulin than in those treated by oral hypoglycemic agents.[34]

Our study has limitations. First, its cross-sectional design precludes the establishment of causal or temporal relations between T2D and elevated liver transaminase levels. Secondly, the performance of liver ultrasound examination was done only in patients with abnormal ALT levels rather than the entire patient sample. This probably makes our prevalence figure an underestimate as many earlier researchers have documented that fatty liver could be encountered even in people with normal ALT and AST levels.[33,35,36]

The prevalence of elevated ALT levels was 10.4% in our study population and ALT elevation was found to be more common in men than to women. There was also a significant association of elevated ALT levels with the duration of diabetes, lower HDL levels and the presence of fatty liver on ultrasound examination. Other risk factors for elevated ALT were younger age and larger waist circumference. Patients on insulin appeared to have lower ALT readings. These findings necessitate interference by lifestyle modification and early therapeutic measures to control risk factors, especially obesity, in younger diabetics which might help to prevent chronic liver disease.

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original article

Endothelial nitric oxide synthase gene Glu298Asp polymorphism in patients with coronary artery disease

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BACKGROUND AND OBJECTIVES: Endo-derived nitric oxide (NO) is synthesized from L-arginine by endothelial nitric oxide synthase (NOS3). Since reduced NO synthesis in endothelial cells has been implicated in the development of coronary atherosclerosis, we investigated the association of NOS3 gene polymorphisms and coronary artery disease (CAD) in an Iranian population.

SUBJECTS AND METHODS: We studied the NOS3 gene Glu298Asp polymorphism in 241 CAD patients with positive coronary angiograms (i.e., >50% stenosis affecting at least one coronary vessel) in Shahid Rajaee Heart Hospital and 261 control subjects without a history of symptomatic CAD. The NOS3 gene polymorphism was analyzed by polymerase chain reaction and restriction fragment length polymorphism. Lipid profile and other risk factors were also determined.

RESULTS: The genotype frequencies of Glu298Asp polymorphism for Glu/Glu, Glu/Asp, and Asp/Asp were 61.3%, 32.2%, and 6.5%, respectively, in control subjects, and 46.5%, 42.7%, and 10.8% in CAD patients, respectively. The genotype frequencies differed significantly between the two groups (P=.003). The frequencies of the Asp alleles were 32.2% and 22.6% for CAD patients and control subjects, respectively; the difference between the two groups was statistically significant (P=.001; odds ratio=1.6). Plasma lipids, except HDL-C, were also significantly increased in the CAD groups.

CONCLUSION: These results suggest that CAD is associated with Glu298Asp polymorphism of the NOS3 gene in our population and that this polymorphism is an independent risk factor for CAD.

Nitric oxide (NO) is synthesized from L-arginine and molecular oxygen by a family of three enzymes: NOS1, NOS2, and NOS3.[1,2] NO is the most powerful endogenous vasodilator known. It can also inhibit the adhesion, aggregation, and recruitment of platelets; inhibit vascular smooth muscle cell migration and growth; regulate some vessel–platelet interactions; and limit the oxidation of atherogenic low-density lipoproteins.[1] These actions are all mediated by the activation of soluble guanylate cyclase and the consequent increase in the concentration of cyclic GMP in target cells.[3]

The constitutive endothelial nitric oxide synthase (NOS3) is expressed in the endothelium and is encoded by a 26-exon gene located on chromosome 7q35 to 36; the gene, with a total size of 21 kb, encodes an mRNA of 4052 nucleotides.[4,5] The NOS3 gene is expressionally and functionally regulated through multiple regulatory steps and entails several polymorphisms, some of which bear functional consequences.[4]

Reduction in basal NO release is known to predispose humans to atherosclerosis, hypertension, thrombosis, and vasospasm. On the other hand, high circulating NO concentrations, which occur with excessive inducible NOS expression under pathological conditions, are generally toxic.[6] Emerging evidence suggests that coronary artery disease (CAD) is related to defects in the generation or function of NO. Markedly increased NO concentrations are reported to be associated with endotoxic shock and exaggerated inflammatory reactions, which may lead to acute hepatic dysfunction and predispose humans to asthma and cardiomyopathy.[6,7]

In general, NOS3 gene polymorphisms have been reported to act as 'susceptibility genes' in various cardiovascular and pulmonary diseases.[7] GT substitution in exon 7 of codon 298,[8] 4a/b polymorphism in intron 4,[9,10] T-786C mutation in the 5′-flanking region,[11,12] and high numbers of CA repeat in intron 13 of the NOS3 gene are also associated with an excess risk for CAD.[13] Among them, a common variant located in exon 7 (G984T) of the NOS3 gene that modifies its coding sequence (Glu298Asp) has been linked by several groups to the risk for CAD and acute myocardial infarction (MI). On the other hand, other studies failed to show any relationship between the Asp variant and the risk for atherosclerosis.[14] In the present study, we investigated the associations between the occurrence and severity of angiographically defined CAD and the Glu298Asp polymorphism.

SUBJECTS AND METHODS

Patients recruited in this study (n=241; 174 males and 67 females) were those who had undergone coronary angiography because of recent MI or angina. They were selected at Tehran Shahid Rajaee Heart Hospital after being angiographically identified (>50% stenosis affecting at least one coronary vessel) as having CAD. The control group consisted of 261 individuals (187 males and 74 females) within the same age range as the patients; they were selected at the same hospital after screening to eliminate individuals with a history of chest pain, diabetes, hypertension, or any other specific illness. Clinical details of these groups are shown in Table 1. All participants were interviewed and data on smoking habits, blood pressure, lipid profile, and any family history of CAD were recorded. The study protocol was approved by the Ethics Committee of Iran University of Medical Sciences and written informed consent was taken from all patients and healthy individuals.

Blood samples were obtained from all subjects after 12 hours of fasting and placed in EDTA tubes and stored at −70°C until the time of assay. Serum concentrations of triglyceride (TG), total cholesterol, low-density lipoprotein (LDL-C), and high-density lipoprotein (HDL-C) were measured by standard methods (ZiestChem Diagnostics, Iran) in the clinical laboratory of the hospital. Genomic DNA was tracted from peripheral blood leukocytes by a standard method (Roche Blood Kit, Germany). Two oligonucleotide primers (sense) 5′-GAC CCT GGA GAT GAA GGC AGG AGA-3′and (antisense) 5′-ACC TCC AGG ATG TTG TAG CGG TGA-3′ based on the flanking sequences of the exon 7 in the NOS3 gene were used to amplify the corresponding DNA fragment by the polymerase chain reaction (PCR).[15] The reaction was performed in a 25-μl final volume and contained 25 pmol of each primer, 0.2 mmol of each deoxynucleoside triphosphate (Roche, Germany),1.5 U Taq DNA polymerase (Fermentas, Lithuania), 50 mmol/l KCL, 2.5 mmol/l $MgCl_2$, 10 mmol/l Tris-Hcl (pH=8.3), and 400 ng of genomic DNA. The reaction was done according to the following protocol: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 6 min. The 517-bp PCR fragments were digested with *BanII* restriction enzyme for 16 hours at 37°C. The wild-type allele (G) has no *BanII* cleavage site, whereas the PCR product was cleaved into two fragments of 346 and 171 bp in the presence of the T984.

Table 1. Clinical characteristics of the studied population.

	CAD patients	Controls	*P* value
No. of subjects	241	261	
Age (years)	53.32 (9.4)	51.8 (8.9)	NS
BMI (kg/m^2)	27.1 (6.1)	27.5 (19.9)	NS
Smoking n (%)	88 (36.5)	66 (25.3)	.004
Family history n (%)	92 (38)	68 (26)	.002
DBP (mm Hg)	85.4 (13.6)	76 (6.7)	.001
SBP (mm Hg)	129.9 (24.3)	117.5 (139)	.001
Cholesterol (mg/dl)	203.8 (45.6)	188.1 (32.3)	.001
TG (mg/dl)	204 (110.5)	169.5 (87.7)	.001
LDL-C (mg/dl)	116.9 (37.2)	105.6 (33.7)	.001
HDL-C (mg/dl)	46.9 (10.4)	48.3 (10.8)	NS
LDL-C/HDL-C	2.63 (0.93)	2.34 (0.88)	.006

Values are mean (SD); BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TG=triglyceride; LDL-C=LDL cholesterol; HDL-C=HDL cholesterol; NS=non-significant.

All statistical analysis was performed with SPSS v. 11.5. Numeric data are presented as mean (standard deviation). The differences between groups were examined by the chi-square test or an independent *t* test as appropriate. ANOVA was used for comparisons among the three groups. Allele frequencies were estimated by the gene-counting method. The frequencies of the alleles and genotypes were compared between patient and control groups by the chi-square test when appropriate. The odds ratio (OR) and 95% confidence intervals (CI) were also estimated. The chi-square test was used to examine the deviation of genotype distribution from the Hardy-Weinberg equilibrium. Logistic regression analysis was used to assess the independent effect of each risk factor in CAD.

RESULTS

The clinical characteristics of the population are shown in Table 1. Control subjects were matched to the case patients by gender and age. Although BMI and HDL-C showed no significant differences between the patients and the control subjects, systolic blood pressure (SBP) and diastolic blood pressure (DBP), triglyceride, total cholesterol, LDL-C, and LDL-C/HDL-C in patients were significantly higher. The frequency of cigarette smoking and family history of CAD in patients were higher than in controls.

The genotype frequencies of Glu298Asp polymorphism in control subjects were 61.3% for Glu/Glu, 32.2% for Glu/Asp, and 6.5% for Asp/Asp. On the other hand, in CAD patients, the genotype frequencies were 46.5% for Glu/Glu, 42.7% for Glu/Asp, and 10.8% for Asp/Asp (Table 2). Although the distribution of genotypes in both the CAD and the control groups satisfied the Hardy-Weinberg equilibrium, the G894T polymorphism of the NOS3 gene was significantly associated with the presence of CAD in our patients (χ^2=11.5, *P*=.003).

The frequencies of the Glu and Asp alleles were 32.2% and 67.8% for CAD patients and 22.6% and 77.4% for the control subjects, respectively; the differences between the groups were statistically significant (χ^2=11.6, *P*=.001, odds ratio=1.6). We analyzed the frequencies of Glu/Asp and Asp/Asp genotypes in CAD patients and control subjects according to age (age≤55 years and age >55 years) and found that the frequencies of Glu/Asp and Asp/Asp genotypes were significantly higher in patients of age≤55 years(χ^2=30.3, *P*=.000, odds ratio=3.84) (Table 3). The logistic regression analysis revealed that the NOS3 mutant allele, smoking, family history, DBP, and LDL-C were independent risk factors for CAD (*P*=.004, *P*=.001, *P*=.006, *P*=.001, and *P*=.001, respectively), whereas DBP, cholesterol, HDL-C, and TG were not independent risk factors for CAD (Table 4).

DISCUSSION

In addition to established risk factors, genetic risk factors may have important roles in the pathogenesis of coronary atherosclerosis and acute MI. Using the

Table 2. Genotype and allele frequencies of Glu298Asp polymorphism of the eNOS gene in CAD patients and controls.

	CAD patients	Controls	Odds ratio (95% CI)	*P* value	χ^2
Glu298 Asp polymorphism				.003	11.5
Glu/Glu, n (%)	112 (46.5)	160 (61.3)			
Glu/Asp, n (%)	103 (42.7)	84 (32.2)			
Asp/Asp, n (%)	26 (10.8)	17 (6.5)			
Total	**241**	**261**	**1.83 (1.28-2.6)**	**.001**	**111.1**
Glu/Glu, n (%)	112 (46.5)	160 (61.3)			
Glu/Asp+Asp/Asp, n (%)	129 (53.5)	101 (38.7)			
Total	**241**	**261**	**1.6 (1.23-2.2)**	**.0001**	**11.6**
Allele					
Glu (%)	67.8	77.4			
Asp(%)	32.2	22.6			
Total	**100**	**100**			

Table 3. Genotype frequencies of Glu298Asp polymorphism of the eNOA gene in CAD patients and controls in different ages.

	CAD patients	Controls	Odds ratio (95% CI)	*P* value	χ^2
Glu298Asp polymorphism					
Age≤55 years			3.84 (2.4-6.3)	.001	30.3
Glu/Glu, n (%)	36 (28)	104 (60)			
Glu/Asp+Asp/Asp, n (%)	93 (72)	70 (40)			
Total	**129**	**174**			
Age>55 years			0.86 (0.47-1.56)	.6	0.27
Glu/Glu, n (%)	76 (68)	56 (64)			
Glu/Asp+Asp/Asp, n (%)	36 (32)	31 (36)			
Total	**112**	**87**			

Table 4. Multiple logistic regression analysis with forward stepwise selection (Wald).

Variable	B	SE	Wald	df	Significance	Exp (B)
eNOS mutant allele	0.61	0.21	8.3	1	.004	1.84
Cigarrette smoking	0.85	0.23	13.4	1	.001	2.34
Family history	0.64	0.23	7.5	1	.006	1.9
Diastolic blood pressure	0.1	0.012	69.2	1	.001	1.1
LDL-C	0.12	0.003	15.9	1	.001	1.01
Constant	9.7	1.05	84.3	1	.001	0.000

approach of epidemiological studies, it is possible to identify weak susceptible genes in polygenic diseases like coronary heart disease.[16] In the last decade, the potential link between an increasing number of gene variants and coronary heart disease has been analyzed by several investigators. Due to the protective roles of NO against important events during atherogenesis, the NOS3 gene has been identified as also having other roles in deciding susceptibility to coronary heart disease.[17,18]

There are many polymorphisms of the NOS3 gene that have been investigated in relation to CAD. Yashimura et al described a point mutation of guanine to thymine at nucleotide 1998 in exon 7 of the NOS3 gene that resulted in replacement of glutamic acid by aspartic acid at codon 298.[8] Later investigations also described other polymorphisms in the 5′ flanking and some introns of the NOS3 gene. Other studies analyzed the relation of the NOS3 gene variation with the risk of CAD. In an Australian population, Cai et al did not detect any association between NOS3 gene polymorphism and the risk of CAD.[19] Hibi et al showed that patients who are homozygous for the Glu298Asp polymorphism might be genetically predisposed to acute MI; however, this mutation apparently is not related to the severity of coronary atherosclerosis.[15] In contrast, Hingorani et al observed an excess of homozygotes for Asp298 variant among patients with CAD. In comparison to Glu298 homozygotes, homozygosity for Asp298 was associated with an odds ratio of 4.2 (95% CI: 2.3 to 7.9).[10] Lembo et al also detected the association of Glu298Asp polymorphism with the risk of CAD in Italy.[20] Gardemann showed that younger T allele carriers of the NOS3 Glu298Asp gene polymorphism with various coronary high-risk profiles had an increased risk of CAD and/or MI.[21] In some of the other studies, such as that by Nassar et al in Canada,[22] Schomoelzer et al in Australia,[23] and Jaramillo et al in Chile,[24] no associations were found between Glu298Asp polymorphism and the risk of CAD. In contrast, Colombo et al,[14] Kerkeni et al,[25] Cam et al,[26] and Berdeli et al[27] detected a link between this polymorphism and CAD in Italian, Tunisian, and Turkish populations. In our previous study, we found that 4a/b polymorphism was not an independent risk factor for CAD in our population.[28] In the present study, we have shown that Glu298Asp polymorphism is an independent risk factor for CAD in this Iranian population, especially in younger patients (age≤55 years).

The frequency of the Asp allele was approximately 0.23 in our population, which is very similar to that previously reported in a Tunisian population (0.22);[25] and slightly higher than that observed in Turkish (0.17)[27] and Indian (0.15)[29] populations and lower than that in the British (0.31),[10] Italian (0.30),[14] and Canadian populations (0.43).[22]

In conclusion, our results show that genotype frequencies for Glu/Glu, Asp/Glu, and Asp/Asp, and also Asp allele frequency, are significantly different between individuals with and without CAD. We have also shown that the Asp allele of NOS3 Glu298Asp polymorphism and DBP are independent risk factors for CAD. In our study, the sample size was small thus; a larger number of patients and controls needs to be examined to confirm the association between this polymorphism and CAD.

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Saudi Guidelines for Testing and Treatment of Latent Tuberculosis Infection

Joint Statement of the Saudi Thoracic Society, the Saudi Society of Medical Microbiology and Infectious Diseases, the Saudi Association of Public Health, and the Society of Family and Community Medicine

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Pulmonary tuberculosis is a common disease in Saudi Arabia. As most cases of tuberculosis are due to reactivation of latent infection, identification of individuals with latent tuberculosis infection (LTBI) who are at increased risk of progression to active disease, is a key element of tuberculosis control programs. Whereas general screening of individuals for LTBI is not cost-effective, targeted testing of individuals at high risk of disease progression is the right approach. Treatment of those patients with LTBI can diminish the risk of progression to active tuberculosis disease in the majority of treated patients. This statement is the first Saudi guideline for testing and treatment of LTBI and is a result of the cooperative efforts of four local Saudi scientific societies. This Guideline is intended to provide physicians and allied health workers in Saudi Arabia with the standard of care for testing and treatment of LTBI.

Over the last century, the tuberculin skin test (TST) has been used extensively in epidemiological surveys and as a clinical test for the diagnosis of latent *Mycobacterium tuberculosis (MTB)* infection (LTBI) in different populations throughout the world.[1-4] It is mainly used for the identification and treatment of persons infected by *MTB*, who are at high risk of progression to active disease.[4] This strategy has proved to be effective in tuberculosis (TB) control because preventive treatment of latently infected people diminishes the risk of subsequent development of active TB by about 90%.[4-7]

The TST uses a relatively crude mix of antigens from MTB. As a result, false-positive reactions can occur because of previous Bacillus Calmette-Guérin (BCG) vaccination or sensitization to nontuberculous mycobacteria (NTM). This complicates the interpretation of the TST as tuberculin reactions caused by BCG or NTM cannot be distinguished with absolute certainty from those caused by latent TB infection. However, the wealth of epidemiological information has allowed the formulation of recommendations for informed interpretation of the TST in most clinical situations.[4,8] We believe, however, that many practitioners remain reluctant to use the TST, and therefore, do not give therapy for latent TB infection even to patients at high risk of reactivation.

This statement provides the first set of national recommendations for targeted tuberculin testing and treatment regimens for persons with latent tuberculosis infection. We strongly believe that targeted testing and treatment of individuals with latent tuberculosis infection, who are at increased risk of progression to active disease, is a key component of tuberculosis control. Infected persons who are considered to be at high risk for developing active TB should be offered treatment of LTBI, unless there are absolute contraindications for therapy.

This statement by the Saudi Thoracic Society (STS), the Saudi Society of Medical Microbiology and Infectious Disease (SSMMID), and Saudi Association of Public Health (SAPH) and Society of Family and Community Medicine, provides the first national recommendation for targeted tuberculin testing and treatment regimens for person with latent tuberculosis infection in Saudi Arabia.

Prevalence of LTBI in Saudi Arabia

The total number of reported tuberculosis cases in the year 2008 was 3 918 cases in a population of approximately 24 807 273. TB not Tuberculosis in Saudi Arabia affects mainly young and middle-aged individuals; most of them aged between 15 and 44 years old. The incidence of all cases was estimated at 15.8/100 000; the incidence of smear-positive tuberculosis was 8.2/100 000. However, WHO 2007 estimation of the incidence of TB new cases was 46/100 000 population/year, the incidence of new smear positive cases was 21/100 000 population/year and the estimated prevalence of all forms of TB cases was 65/100 000 population/year.[10] Al Kassimi et al in 1993[9] conducted the first comprehensive and nationwide tuberculin survey in the Saudi Arabian general population with urban/rural stratification. Using a definition of a positive tuberculin test of 10 mm or more, 33% of the subjects had a positive TST, and 56% were aged 45 years and older. Given that false positive reactions can occur in almost all populations, the most important determinant of the utility of a TST is the expected prevalence of true latent TB infection. The incidence of smear-positive cases of pulmonary tuberculosis varies widely between countries. Based on the observation by Styblo[9] of the relationship between the incidence of smear-positive pulmonary TB and the annual risk of TB infection (ARI), one can calculate the ARI based on the expected prevalence of latent TB infection in persons of a given age. In Saudi Arabia, the estimated incidence of active TB (all forms) is 46 per 100 000 and the incidence of smear-positive TB is 21 cases per 100 000, which gives an annual risk of infection of 0.35%.[10] Using the Stabylo[9] calculation, the prevalence of LTBI at 10 and 20 years is 3.4% and 6.7% respectively, placing Saudi Arabia in the intermediate prevalence (2%-14%) category.[11]

Administrative technique for the TST

The administration of TST requires proper technique by trained professionals. A standard tuberculin syringe with a 27-gauge needle should be used to inject five tuberculin units of purified protein derivative (PPD) or two tuberculin units of RT-23 intradermally. Usually, the volar or inner surface of the forearm is used. The area of the skin selected for testing should be free of any cutaneous or subcutaneous lesion that could interfere with any interpretation. The injection should be intradermal, and neither too deep nor too shallow. A small wheal measuring approximately 5 mm in diameter should be elicited at the time of injection. If incorrect administration is suspected, a second test dose can be given immediately at a site that is at least 5 cm away from the first one.[4] As a result of its tendency to be adsorbed by glass and plastics, tuberculin materials should never be transferred to secondary containers. It should be administered as soon as feasible after filling the syringe, ideally within 20 minutes. Long-term storage of prefilled syringes is not recommended. Tuberculin material should be kept refrigerated but not frozen, and most importantly, kept protected from light.

Reading the TST

The tuberculin reaction is a classic, delayed type, hypersensitivity immune reaction. This means that the reaction begins within 24 hours, is maximal between 48 and 72 hours after injection, and then wanes over the next few days. Reading is done between 48 and 72 hours after administration.

The transverse diameter of the induration, but not erythema, is demarcated using the ball-point technique. The induration is measured and recorded in millimeters. If there is no reaction, the size should be recorded as 0 mm and not simply as "negative". Similarly readings of "doubtful" or "positive" are discouraged, particularly in individuals in whom repeated testing is done (see below).[4]

Adverse reactions to tuberculin skin testing are uncommon. Local allergic reactions to tuberculin or its components can occur in 2% to 3% of those tested usually in the form of a rash on the arm. Generalized rash has also been observed. These reactions begin shortly after injection and disappear within 24 hours. Severe blistering is possible, for which topical steroids are frequently given, although there is no evidence of their efficacy. The blistered area should be covered with a dry dressing and the patient advised to keep it clean and not to scratch it.[4]

Interpretation of the TST

False-negative TST

Many factors can cause false-negative tests including viral, bacterial, or fungal infections. The most important cause of a false-negative TST is HIV infection. In HIV-infected persons, the likelihood of a false-negative TST is inversely proportional to the CD4 count: it is uncommon at CD4 counts above 500/mL, and almost universal when the CD4 count is less than 200/mL. Saudi Arabia has a low prevalence of HIV infection of less than 0.01%.[12,13] HIV infection is therefore not an important cause of a false-negative TST in Saudi Arabia.

It is important to note that patients with active TB can have false-negative tests at the time of diagnosis, particularly those with more advanced disease.[4] Anergy testing with control antigens to which most normal adolescents and adults should react, such as mumps and *Candida*, have been used to determine whether people can have true- or false-negative tuberculin reactions. However, anergy testing is not routinely recommended in persons who are HIV-infected or otherwise immunocompromised because of the evidence that this testing is unreliable.[4] Other causes of false-negative TSTs are presented in Table 1.

False-positive TST

BCG vaccination was started in 1964 in Saudi Arabia and a 95.9% coverage rate was achieved by 2007.[14] The BCG vaccine is given at birth without any booster doses. A case-control study in Saudi Arabia determined the protective effect of the BCG vaccine using the same BCG strain (freeze-dried glutamate BCG, Japan laboratory Tokyo).[15] This study demonstrated an 82% protective effect in the 5-14-year-old age group, but no protective effect 25 years after vaccination.[15]

Table 1. Causes of false-negative tuberculin skin testing

Technical factors: (potentially correctable)

- Defective antigens because of improper storage (exposure to light or heat) or manufacturing
- Contamination, improper dilution
- Injection of too little tuberculin, or too superficial, or too deeply (should be intradermal)
- Prolonged storage within the syringe before administration (more than 20 minutes)
- Inexperienced or biased reader
- Error in recording

Biological factors: (not correctable)

- Viral, bacterial, or fungal infections
- HIV (especially if CD4 count <200)
- Live virus vaccination within the past two months
- Metabolic derangement, protein depletion, chronic renal failure, severe malnutrition, stress (surgery, burns)
- Concurrent use of immunosuppressive drugs: (corticosteroids, TNF inhibitors, and others)
- Very young <6 months or elder
- Diseases of lymphoid organs: (lymphoma, chronic lymphocytic leukemia, sarcoidosis)

In several other studies following BCG vaccination in infancy, tuberculin reactivity was seen to wane rapidly so that there was little discernible effect by the age of five years. However, those vaccinated at a later age have larger TST reactions that wane more slowly, with 15% to 25% of the reactions persisting beyond ten years.[16-22]

A meta-analysis including more than 24 studies found that only 1% of patients who received BCG during infancy were TST positive if tested more than ten years after BCG vaccination. However, there was a greater and more long-lasting effect on TST if BCG was given later in life, such as during the primary or secondary school-going years.[23] Most of the international guidelines recommend ignoring BCG's effect on the interpretation of TST in persons at increased risk of developing active TB.[4]

Nontuberculous mycobacteria

Nontuberculous mycobacteria (NTM) are another major cause of false-positive TST.[4,20,24] The prevalence of NTM varies considerably between regions, countries, and even within countries. There are few studies of the prevalence of NTM in the Middle East. Two recent hospital-based studies in Kuwait and Saudi Arabia identified NTM in 18 of 325 (5.9%) and 70 of 286 (24.5%) specimens, respectively.[25,26] An earlier similar study from Saudi Arabia gave an intermediate figure of 9%.[27] A nationwide population-based survey, however, revealed a much lower figure of 0.004%.[9] A recent meta-analysis involving more than 18 studies found that NTM is not a clinically important cause of false-positive TST, particularly in areas of high TB prevalence.[28]

Serial tuberculin testing (conversion or boosting)

When repeated tuberculin testing has been performed, a substantial number of individuals may manifest an increased tuberculin reaction on their second test in the absence of any obvious exposure. It is now realized that this reflects an anamnestic response representing the restimulation of previously acquired immunity from earlier exposure.[29] This phenomenon, termed "boosting", results from a previous mycobacterial exposure, including remote BCG vaccination, nontuberculous mycobacterial exposure, or remote infection with MTB.[4,29]

It is important to distinguish boosting from the phenomenon of tuberculin conversion which occurs after an initial tuberculin infection. This is because the risk of developing active tuberculosis in persons who manifest the "boosting" phenomenon is actually lower than those with an initial positive TST, and much lower than in persons with tuberculin conversion.[29] In persons with conversion, the risk of disease is between 5% and 20% over the next two years alone.

It is difficult to distinguish the phenomena of boosting and conversion simply on the basis of size, although if the second reaction is >15 mm, it is most likely to be conversion. If the second TST is performed soon (within two weeks) after the first and there is no intervening exposure, then boosting is most likely the cause for a positive TST. If the second test is performed months to years after the first, and there has been exposure to MTB (such as contacts of active cases), then the cause for a positive TST is considered to be recent TST conversion, indicating TB infection.[29]

In some clinical situations, it may be necessary to repeat the TST on a periodic basis. An initial two-step testing protocol is recommended in this situation. The most common situation is when persons enter an environment where there might be a risk of exposure to TB (e.g., persons entering professions or facilities with an increased risk of occupational exposure such as health care or prisons).[4,29]

If the initial TST is negative (<10 mm), then a second test should be performed 1 to 4 weeks later to elicit the booster phenomenon.[4] Boosting can be seen after intervals as long as two years, but this phenomenon is maximal if the two tests are between one and four weeks apart, and a shorter interval is always more practical. If the second tuberculin test is negative (<10 mm), the individual can be considered to be truly negative. If on subsequent retesting, the tuberculin test is positive, this can be considered as a true conversion. This is strong evidence of new infection and should therefore be treated.[29]

A TST conversion is defined as a TST reaction of 10 mm or more plus as an increase of 6 mm or more of induration from the previous TST results within a two-year period, regardless of age.[4,29,30] The ATS/CDC/IDSA definition of conversion requires an increase of 10 mm or more from the previous TST result.[4,7] This more stringent criteria will be more specific but less sensitive to detect conversion.

Indications for TST and who should be treated

Routine screening of asymptomatic subjects is not recommended.[4,31] The risk-benefit ratio for treating LTBI depends principally on whether the individual is truly infected and is at substantial risk of developing active TB disease. Therefore, TST screening for LTBI should be reserved for those groups with a high risk of recent infection, or who are at high risk of progression from LTBI to TB disease as outlined in Tables 2 and 3.

Normal healthy individuals with LTBI have an annual risk of 0.1% (1 per 1000) of developing active TB.[32] Depending on the individual's clinical condition, the annual risk of disease, if infected, can range from more than 10% for HIV patients to 1 to 2% for patients on hemodialysis.[33-35]

High-risk individuals are those whose risk for reactivation is at least six times higher than for normal, healthy individuals.[33,36-44] Moderate-risk individuals are those whose risk for reactivation is 3 to 6 times higher than for normal, healthy individuals.[45-52] Low-risk individuals are those at slightly increased risk for reactivation and whose risk is 1.5 to 3 times higher than for normal, healthy individuals.[53-55] Table 4 shows the estimated risks for TB relative to healthy individuals in various conditions. Defining a positive tuberculin reaction is dependent on the size of the TST: ≥5 mm is considered positive in high-risk individuals whereas ≥10 mm is considered positive in moderate-risk individuals. For persons at low risk for TB and with high risk of exposure to NTM (for whom tuberculin testing is not generally indicated), ≥15 mm of induration is considered positive. Treatment for LTBI is contraindicated in patients with active TB disease and in patients with severe liver disease.[4]

The principle indications and precautions in latent tuberculosis testing and treatment

1. The goal of testing for latent tuberculosis infection is to identify individuals who are at risk of new infection, and to identify individuals at increased risk of reactivation due to the biological factors listed in Table 1.
2. All people who are considered high risk should be tested, regardless of BCG history. A history of BCG vaccination should not be considered when deciding whether to perform TST or not. Furthermore, the effects of BCG on TST should be ignored in high-risk individuals.
3. NTM is not a clinically important cause of false-positive TST, particularly in Saudi Arabia, and should not be considered in the interpretation of TST.[9,25-28]
4. Patient age is of some importance in the decision to test for LTBI. Testing is not recommended for individuals ≥65 years unless they are at high risk for reactivation.[56]
5. It is essential to exclude active TB disease in persons with newly detected latent TB infection. Treatment

Table 2. Indications for tuberculin skin testing.

A. Individuals with increased risk for new infection: (all patients should be tested regardless of age)

- Close contacts of persons with active pulmonary TB
- Persons whose tuberculin skin tests have converted to positive (≥10 mm induration and >6 mm increase) within the past two years
- Persons who live or work in clinical or institutional settings where TB exposure may be likely (e.g., hospitals, prisons, nursing homes, microbiology labs)

B. Individuals with clinical conditions associated with increased risk of reactivation:

1. High risk (all patients should be tested regardless of age)

- Persons with HIV infection
- Persons who never received antituberculosis therapy with abnormal chest x-ray with apical fibronodular changes typical of healed TB (not including granuloma)
- Persons with certain medical conditions (e.g., silicosis, chronic renal failure on dialysis
- Transplant, lymphoma, leukemia chemotherapy

2. Moderate risk (only patients less than 65 years of age should be tested)

- Diabetes mellitus
- Persons receiving immunosuppressive therapy (e.g., prolonged corticosteroid therapy [the equivalent of >15 mg/day of prednisone for one month or more], TNF-α blockers)
- Children four years of age

3. Low risk: test not indicated

Table 3. Target for tuberculin skin testing and who should be considered for treatment.

Tuberculin skin test reaction size, (mm induration)	Situation in which reaction is considered positive
TST≥5 mm	HIV seropositive Close contact with positive AFB in the sputum Fibrotic changes on chest radiograph consistent with prior untreated tuberculosis Organ transplants and other immunosuppressed patients (e.g., persons receiving the equivalent of 15 mg/day of prednisone for at least 30 days or more)
TST≥10 mm	Injection drug use Residents and employees in high-risk settings: nursing homes, and other long-term care facilities, hospitals Persons at increased risk of developing active tuberculosis: those with silicosis, diabetes mellitus, chronic renal failure, leukemia, lymphoma, cancer of the head, neck or lung, weight loss of more than 10% of ideal body weight, gastrectomy, jejunoileal bypass Children younger than four years of age
TST≥15 mm	Persons with no risk factors for tuberculosis
Contraindications for treatment	Any clinical, radiological, or bacteriological evidence of TB disease

for LTBI should not be initiated until active disease has been excluded. Medical examination and chest x-ray should be done to rule out tuberculosis disease for any individual with a newly identified positive test. Persons with radiographic signs or symptoms that are suggestive of active TB should undergo sputum smear microscopy and culture to rule out active TB.

5.1 If the radiograph is normal and the patient has no symptoms or physical findings consistent with TB, treatment of LTBI may be indicated.

5.2 If the radiograph is normal but the patient has a clinical presentation consistent with TB, further workup is indicated and treatment of LTBI should be delayed until active TB has been ruled out.

5.3 If the radiograph is abnormal and consistent with TB, specimens of sputum (using sputum induction if necessary) should be obtained. Starting treatment pending the culture results or waiting for culture results before starting therapy, is a clinical judgment dependent on benefit-risk considerations.

5.4 If a radiograph is abnormal but was taken more than three months prior to evaluation, a new radiograph should be performed at the time of evaluation.

5.5 If the abnormality on the chest radiograph is of questionable significance, consultation with an expert is recommended.

Aims of and recommended regimens for LTBI treatment

The term, 'treatment of LTBI' has been adopted to highlight the fact that the patient is considered to be infected with live (although dormant) bacilli, which could cause active TB disease in the future, and that there is effective treatment available for this infection.

1. Aim of treatment

Treatment of LTBI is intended to prevent the development of active TB disease. In the past, this has been referred to as 'chemoprophylaxis' or 'preventive therapy'. Latent tuberculosis infection is associated with a low burden of organisms; therefore, treatment of latent infection requires fewer drugs than active disease to decrease the risk of developing active tuberculosis without creating drug resistance. In most of the cases, use of a single antituberculosis agent is sufficient for the treatment of latent infection, but not for active disease.

2. Recommended regimens for treatment

i. Four basic regimens are currently recommended (Table 5).
ii. Completion of therapy is based on the total number of doses administered, not the duration of therapy alone.
iii. Treatment interruptions of more than two months require another evaluation to exclude active TB before restarting therapy.

a. Isoniazid alone—preferred regimen

Daily INH for 9 months is the preferred regimen for the treatment of LTBI.[4,57-59]

- Daily isoniazid INH for a 9 month regimen for children and adolescents (up to age 18 years).
- Daily INH for a nine-month regimen for HIV-infected persons or persons suspected of having HIV infection.
- Daily INH for a nine-month regimen for immunocompetent adults.

Intermittent (twice weekly) INH for nine months has not been compared directly with daily administration but is considered to be an acceptable alternative, provided all doses of therapy are directly observed.[4]

b. Six months INH alone—acceptable alternative:

- INH may be given for six months if there are any concerns about side effects or adherence.

c. Rifampin for 4 to 6—acceptable alternative

This regimen should be reserved for those individuals who cannot tolerate INH or for persons exposed to cases with resistance to INH, but susceptible to RIF.

- Rifampin (RIF) alone for four months is generally recommended for adults.
- Rifampin alone for six months is generally recommended for children.[60]
- Rifabutin (RFB) may be substituted for rifampin in the above regimens in situations where rifampin cannot be given, such as in HIV-infected persons taking protease inhibitors or non-nucleoside reverse transcriptase inhibitors.[4]

d. Rifampin and pyrazinamide for two months—NOT recommended

This regimen is not recommended due to fatal hepatotoxicity. Therefore, it should generally NOT be offered as initial therapy to persons with LTBI for either HIV-negative or HIV-infected persons.

Table 4. Relative risk for developing active tuberculosis by selected clinical conditions

Risk factor	Estimated risk for TB relative to persons with no known risk factor	References
High risk		
Acquired immunodeficiency syndrome (AIDS)	110-170	33, 36
Human immunodeficiency virus infection (HIV)	20-74	37, 41
Silicosis	30	40, 88
Chronic renal failure on hemodialysis	10.0-25.3	35, 38, 39, 89
Transplantation (related immunosuppressant therapy)	20-74	76-79
Jejunoileal bypass	27-63	90, 91
Carcinoma of head or neck	16	92, 93
Recent TB infection (≤2 years)	15	42, 43
Abnormal chest x-ray with apical fibronodular changes typical of healed TB (not granuloma)	6-19	44, 71, 94
Moderate risk		
Tumor necrosis factor (TNF)-α inhibitors	1.5-4	45, 46, 95
Treatment with glucocorticoids	4-9	47
Diabetes mellitus (all types)	2-3.6	48-51
Young age when infected (≤4 years)	2.2-5	52, 53, 72, 73
Low risk		
Underweight (<85% of ideal body weight); for most individuals, this is equivalent to body mass index (BMI) ≤20.	2-3	53
Cigarette smoker (1 pack/day)	2-3	54, 55
Chest x-ray with solitary granuloma	2	94, 96

3. Monitoring of treatment

- Education about adverse effects associated with the treatment of LTBI, with advice to stop treatment and promptly seek medical evaluation if serious adverse effects occur.
- Follow-up evaluation at least monthly, including careful questioning and a brief physical examination to assess for evidence of hepatitis or other adverse effects, symptoms of TB disease, and adherence to the regimen.
- All persons receiving treatment for LTBI should be monitored clinically for symptoms and signs of adverse drug reactions, beginning at the first initial

Table 5. Recommended drug regimens for the treatment of LTBI.

Drug	Interval and duration	Oral dosage (maximum)	Criteria for completion	Comments
Isoniazid	Daily×9 mo	Adult: 5 mg/kg (300 mg) Child: 10-20 mg/kg (300 mg)	270 doses within 12 mo	INH daily for 9 months is the preferred regimen for all persons and the only regimen for persons with fibrotic lesions on CXR
	Twice-weekly by DOT×9 mo	Adult: 15 mg/kg (900 mg) Child: 20-40 mg/kg (900 mg)	76 doses within 12 mo	Use twice-weekly regimen only if daily regimen not feasible. DOT must be used with twice-weekly dosing
Isoniazid	Daily×6 mo	Adult: 5 mg/kg (300 mg)	180 doses within 9 mos	Use ONLY if preferred regimen not feasible. Not recommended for HIV-infected persons† those with fibrotic changes on CXR, or children aged <18 years. DOT must be used with twice-weekly dosing.
	Twice-weekly by DOT×6 mo	Adult: 15 mg/kg (900 mg)	52 doses within 9 mo	
Rifampin	Daily×4 mo	Adult: 10 mg/kg (600 mg) [Child: 10-20 mg/kg (600 mg) see comments]	120 doses within 6 mo	Use ONLY if preferred regimen not feasible. Not recommended for persons with fibrotic changes on CXR. Not recommended for persons aged <18 years, unless exposed to INH-resistant, RIF-susceptible TB. For HIV-infected persons, most protease inhibitors or non-nucleoside reverse transcriptase inhibitors should not be administered concurrently with RIF. Consult web-based updates for the latest specific recommendations.

RIF + PZA: Generally should not be offered for treatment of LTBI.

visit and at least monthly thereafter.

- Routine laboratory monitoring of liver function tests (LFTs) is recommended for persons with baseline abnormalities in LFTs or other risk factors for drug-induced hepatitis. Additional testing is indicated if symptoms or signs indicative of hepatitis develop.
- Indications for baseline LFTs, including serum bilirubin and either aspartate aminotransferase (AST) or alanine aminotransferase (ALT) are as follows:
  1. Person with viral hepatitis or for whom complete hepatitis serology results are unknown
  2. HIV infection
  3. Pregnancy or less than three months postpartum
  4. History or initial evaluation indicative of hepatitis or cirrhosis
  5. Regular alcohol use
  6. Use of potential hepatotoxic medication
- Indications for monthly LFTs, including serum bilirubin and either AST or ALT, are as follows:
  i. Abnormal baseline LFT
  ii. Chronic liver disease
  iii. Regular alcohol use
  iv. Current use of hepatotoxic drugs
- We recommend withholding INH if serum transaminase concentrations exceed three times the upper limit of the normal range when accompanied by symptoms, or five times the upper limit of the normal range in asymptomatic patients.

Treatment of latent tuberculosis in special situations

Pregnancy/lactation

Pregnancy has minimal influence on the pathogenesis of TB and there is no evidence to suggest a greater risk of progression of LTBI to active disease during pregnancy.[4] The treatment of LTBI during pregnancy remains controversial. However, under ordinary circumstances, physicians should delay treatment until two to three months after delivery.[4] However, for pregnant women who are HIV-positive or at high risk of progression to active disease and to prevent its consequences on the mother and the fetus, initiation of therapy should not be delayed on the basis of pregnancy alone, even during the first trimester.[4] INH can be given to pregnant women and is not toxic to the unborn child, even during the first four months of gestation.[4,7,61-66] Pregnant women taking INH should receive vitamin B6. Breastfeeding is not contraindicated when the mother is being treated for LTBI.[4]

Treatment of HIV-infected persons

Recommendations for HIV-infected adults are in general similar those for HIV-negative adults, although the quality of evidence and strengths of the recommendations vary.[4,67-70] If isoniazid is chosen for treatment of LTBI in persons with HIV infection, nine months of therapy is recommended rather than six months. In addition, rifampin should generally be avoided in persons who are taking protease inhibitors (PIs) or NNRTIs.[4,7] However, the management of persons co-infected with HIV and LTBI can be highly complex. Our recommendation is that management should be attempted in consultation with physicians who are experts in the treatment of TB and HIV.

Persons with fibrotic lesions/suspected disease

Patients who have a chest radiograph demonstrating upper lobe fibronodular changes or scarring compatible with previous TB and a positive TST (>5 mm) without evidence of active disease and no history of treatment for TB, are considered at high risk of reactivation and should receive treatment.[4,7,44,71] Persons with evidence suggestive of healed, primary TB (i.e., calcified solitary pulmonary nodules, calcified hilar lymph nodes, and apical pleural capping) have only slightly increased risk for TB (about double that of the healthy). Their risk for TB and need for treatment of LTBI should be determined by considering other risk factors.[4]

Renal failure

Patients with chronic renal failure are at a high risk of reactivation (relative risk, 10.0-25.3) and all patients with positive reaction are recommended for therapy.[38,39] However, anergic reaction is common among patients requiring hemodialysis, even in patients resident in endemic areas, so that TST may not be very sensitive to detect LTBI. In the absence of randomized trials that are specific for this population, nine months of isonizid (INH) is recommended.[4]

Children and adolescents

Infants and young children (i.e., those younger than five years of age) with LTBI are at high risk of progression to active TB. If untreated, they have up to 40% likelihood of developing active TB.[4,52,72,73] In particular, young children in contact with adults with active TB (i.e., household contacts) are at high risk for developing TB disease and should therefore be targeted for LTBI therapy (after ruling out active TB). The risk of progression decreases gradually through childhood. Infants and young children are more likely than older children and adults to develop life-threatening forms of TB, especially meningeal and disseminated disease. INH therapy for LTBI is more effective for children than adults, with risk reduction of 70-90%. Infants, children, and adolescents generally tolerate INH better than adults.[4,72,73]

The only recommended regimen for the treatment of LTBI in HIV-negative children is a nine-month course of INH as self-administered daily therapy or by DOT twice weekly.[4] Routine administration of pyridoxine is not recommended for children taking INH, but should be given to (1) breastfeeding infants, (2) children and adolescents with diets likely to be deficient in pyridoxine, and (3) children who experience paresthesias while taking INH. RIF alone has been used for the treatment of LTBI in infants, children, and adolescents when INH could not be tolerated, or when the child has had contact with a patient infected with an INH-resistant but RIF-susceptible organism. If RIF alone is used, then a six-month treatment regimen is recommended.[4,72,73]

Liver disease

Patients with chronic liver disease with LTBI pose a special problem, as all available regimens are potentially hepatotoxic. Four months of RIF may be safer although the efficacy of such a regimen has not been established.[74,75] However, frequent clinical and laboratory monitoring for drug side effects is prudent.

Solid organ transplantation

Solid organ transplantation is associated with a very high risk of LTBI reactivation to active TB. The incidence of infection among such patients is estimated to be 20-74 times that for the general population.[76-79] They should be treated for LTBI if their TSTs are positive. Safety of INH remains a concern, especially in liver transplant recipients, but is generally safe as long as pretreatment liver transaminase levels are normal. Common strategies include INH for nine months or RIF for four months. Patients should be treated during the pretransplant period, if possible.

Contacts of patients with drug-susceptible tuberculosis index case

Persons who are contacts of patients with drug-susceptible TB and who have positive tuberculin skin-test reactions should be treated with one of the recommended regimens listed above.[4] In addition, some tuberculin-negative close contacts who are at high risk to develop severe active disease (e.g., children younger than five years of age) should be treated and another skin test performed a few weeks after contact has ended. If the repeat skin test is negative, the treatment should be discontinued.[31] Immunosuppressed persons, including those with HIV infection, who are close contacts of persons with active TB, should also receive treatment, even if repeat skin testing remains negative.[4,31]

Contacts of index cases with isoniazid-resistant tuberculosis

For TST-positive contacts of index or source cases with INH-resistant TB, we recommend treatment with four months of RIF; this appeared to be effective for tuberculin converters in two outbreaks of INH-resistant TB.[80,81] In situations in which RIF cannot be used, rifabutin can be substituted.

Contacts of patients with a multidrug-resistant tuberculosis index case

Treatment of LTBI after possible exposure to multidrug-resistant TB has not been evaluated in a randomized trial. For those who are at high risk of progression to active TB, preventive therapy with two drugs to which the organism is expected to be susceptible is recommended. Ideally, the selection of drugs should be guided by in vitro susceptibility test results from the isolate to which the patient was exposed and is presumed to be infected.

For persons who are likely to be infected with MDR-TB and are at high risk of developing TB, treatment should be with at least two active agents. Pyrazinamide (PZA) and ethambutol (EMB) or PZA and fluoroquinolones (FQN) have been recommended for 6 to 12 months.[82] However, the latter regimen is very poorly tolerated;[83] hence, we recommend an FQN (levaquin or moxifloxacin) and EMB as the preferred regimen for adults. PZA and EMB are recommended for 9 to 12 months for children because FQN use is contraindicated in pediatric patients.[4,31] All persons with suspected latent MDR-TB infection should be followed for at least two years, regardless of the treatment regimen. When FQN and EMB cannot be used, experts recommend using a combination of two other drugs to which the infecting organism is likely to be susceptible.

Use of interferon release assays

A major advance in recent times has been the development of T-cell-based interferon-gamma release assays (IGRAs).[84,85] IGRAs are in vitro tests that are based on interferon-gamma (IFN-γ) release after stimulation by antigens (such as early secreted antigenic target 6 [ESAT-6] and culture filtrate protein 10 [CFP-10]) which are more specific to MTB than the purified protein derivative (PPD). Two IGRAs are currently available as commercial kits that are US Food and Drug Administration (FDA)-approved, and marked for use in Europe: the QuantiFERON-TB Gold® In-Tube (QFT) assay (Cellestis Ltd., Carnegie, Australia) and the T-SPOT.TB® assay (Oxford Immunotec, Abingdon, UK). Of the two commercial tests, the QFT assay is simple and easy to use because of its ELISA format, and less expensive.

There are many potential advantages of these new tests. They are standardized and quality-controlled laboratory tests that provide results in a single patient visit. They do not have a 'booster' effect, and can be repeated without the need for two-step testing. They have excellent specificity and are unaffected by BCG and NTM (estimated specificity >98%).[84,85] The high specificity of IGRAs is likely to be useful in BCG-vaccinated individuals, particularly in countries where TST specificity is compromised by BCG vaccination after infancy or by multiple BCG vaccinations.

Sensitivity of IGRAs and TST is not consistent across tests and populations, but IGRAs appear to be at least as sensitive as the TST (estimated with active TB as the surrogate reference standard).[84,85] However, IGRAs should not be used to diagnose active TB because they cannot distinguish between LTBI and active disease.

IGRAs have some disadvantages, including higher material cost, the need for an equipped laboratory, and a requirement to draw peripheral blood. Evidence is still limited on the prognostic (predictive) value of these tests, and their added value in TB diagnosis and control. Furthermore, the interpretation of IGRA conversions and reversions is unclear, and evidence is rather limited in children and immunocompromised populations. Many studies on IGRA are ongoing in this area to properly define the role of IGRA in day-to-day clinical practice.[86]

Use of IGRA for the diagnosis of latent TB infection

The use of IGRAs is steadily increasing in low or intermediate incidence countries. More than a dozen countries (e.g., USA, UK, Canada, Germany, France, Switzerland, Japan, Netherlands) now have at least one guideline or statement on the use of IGRAs. There is considerable diversity in how various countries currently recommend and use IGRAs. For example, the CDC guidelines for the USA recommend using either TST or IGRA for LTBI testing. In contrast, many countries (e.g., UK, Canada, Spain, Italy) recommend a two-step approach where TST is done first, followed by IGRA. Globally, the two-step approach seems to be the most popular and possibly a very cost-effective strategy. However, because of the potential boosting effect of TST on IGRA results,[87] it may be best to collect blood for IGRA at the time the TST is read (i.e., within three days of placing PPD).

Recommendations

1. IGRAs should not be used to diagnose active TB in adults. In children, they may be used as an adjunct test, in combination with TST, chest x-ray, and microbiological investigations. A negative IGRA alone should not be used to rule out active TB.
2. In BCG-vaccinated individuals (adults and children), IGRAs may be used to confirm a positive TST result (i.e., to check if the TST result is a false positive). If a positive TST result is confirmed by a positive IGRA result, LTBI treatment should be initiated after ruling out active TB.
3. In immunocompromised patients, if a false-negative TST result is suspected, IGRAs may be used to rule out LTBI.

Conclusions

Saudi Arabia is a country of medium prevalence for TB infection. The TST remains a useful tool in the identification of subjects with latent TB infection. NTM infection and BCG vaccination (routinely given in infancy) do not interfere with the interpretation of the test in adolescents and adults. Patients at high risk of developing active TB should be treated with the standard regimens advocated in this statement after due care is taken in excluding active TB disease.

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perspectives

Trauma Care Systems in Saudi Arabia: An Agenda for Action

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Saudi Arabia is undergoing a rapid population growth that along with improved socioeconomics has led many individuals to own a car or even a number of cars per family, resulting in a greater number of vehicles on the roads. The reduced focus on good public transportation systems and the dependence on cars for transportation have created a diversity of drivers who are unfamiliar with the local driving rules and lack the basic skills for safe driving. This is in addition to some young drivers who frequently violate traffic laws and tend to speed most of the time. This unplanned expansion in road traffic has resulted in more car accidents, injuries, disabilities, and deaths. Accompanying that is an increased socioeconomic burden, depletion of human resources, emotional and psychological stress on families, and a strain on healthcare facilities. If this continues without prompt intervention, it will lead to increased insurance premiums and may become unmanageable. To minimize this impact, a national or regional multidisciplinary trauma system has to be developed and implemented. A trauma system is a preplanned, comprehensive, and coordinated regional injury response network that includes all facilities with the capability to care for the injured. Essential components of the system include trauma prevention, prehospital care, hospital care, rehabilitation, system administration, trauma care education and training, trauma care evaluation and quality improvement, along with the participation of society. Research has documented a significant decrease in morbidity and mortality from trauma after the implementation of such systems, depending on their efficiency. The purpose of this review is to discuss the problem of road traffic accidents in this country and address the trauma care system as an effective solution.

Urban development in Saudi Arabia resulting from the oil boom and the sharp rise in living standards has brought about dramatic changes to the road network and an increase in the number of cars. This rather abrupt change has contributed to an increase in the number of road traffic accidents (RTAs) and a multiple-fold rise in the number of injuries and deaths over the last few decades.[1,2] National efforts are not up to expectations for coping with these problems. These efforts have been mostly directed toward limited changes in traffic laws and improving roads, not addressing the root causes. The legal regulatory framework is not well-enforced and minimal attention is paid to changing driver behavior, which is considered the most important and most difficult factor to control.[3,4] Many programs and campaigns have failed to re-educate drivers, probably because they were targeting the wrong audience. The way people live is the way they drive.[5] To bring about positive behavioral changes, attention should be directed towards young people before they are actually allowed to drive.[6,7] The aim of this review is to discuss the magnitude of the problem of road traffic accidents (RTAs) in Saudi Arabia and suggest the establishment of a trauma care system as a solution to improve trauma outcomes.[8-10]

Socioeconomic and medical effects of RTAs

Road traffic accidents (RTAs) or motor vehicle accidents (MVAs), when fatal or otherwise, are a socioeconomic burden on the victims and their families and on the hospitals and rehabilitation centers. Trauma deaths tend to deplete the pool of human resources as the majority of the victims are working males.[11] It is difficult to estimate the economic cost of RTAs as it entails medical care, rehabilitation services, and the loss of productivity as a result of absenteeism or disability.[12] In the city of Jeddah alone, the cost of road fatalities in 1987 was estimated to be 648.7 million Saudi Riyals (USD $172.5 million).[13] A conservative estimate of the annual cost of RTAs to the country is 21 billion Saudi Riyals (USD $5.6 billion), an estimated loss of 2.2% to 9% of the national income.[1] The loss of an earning member of a family leads to a change in household dynamics, causing financial and emotional losses.[1,14] Some of those who lose their ability to earn a livelihood on account of a disability are burdened with their own healthcare, and a number resort to selling most of their assets and may even end up in bankruptcy.[14] About 3000 people worldwide die every day from road traffic injuries.[14] Low- and middle-income countries account for about 85% of the deaths and for 90% of the annual disability-adjusted life years (DALYs) lost because of road traffic injury.[14] Trauma is the leading cause of mortality and morbidity in young age groups in Saudi Arabia[1] with RTAs accounting for 80% to 85% of these traumas.[15] In 1986, the death rate from RTAs was 26.5 per 100 000 of the resident population.[13] It has been estimated that in this country, one person is killed and four are injured every hour.[1] The traffic data for 2005 have shown that the number of RTAs in Saudi Arabia was 283 684 with 5883 deaths; half of them were below 30 years of age, and around 60% were Saudis.[16] RTAs are most common in the Makkah region followed by the Eastern province.[16] The highest incidence of RTAs was found on four major roads: Dammam-Riyadh road, Dammam-Jubail road, Riyadh-Taif road, and Riyadh-Qassim road.[17] At any given time, about one-fifth of Ministry of Health hospital beds are occupied by road traffic victims.[1] Many of these patients have residual disabilities requiring long-term rehabilitation care, which is suboptimal in most regions. Approximately 74% of all cases of hemiplegia, paraplegia, and quadriplegia in Saudi Arabia are due to road traffic accidents.[1] Head and facial injuries account for 30% of all injuries and cause 26% of deaths.[1] Musculoskeletal and central nervous system injuries require the highest care and contribute to prolonged hospital stay.[18] Blunt abdominal trauma is a common injury, especially in children, with the liver and spleen being the most frequently injured solid organs in 91% of cases.[19] Blunt trauma from RTAs is the most frequent cause of chest injuries in children, with the lung being the most commonly injured intrathoracic structure.[20] The relative compressibility of the child's thorax accounts for the severe intrathoracic injuries without rib fracture following a crushing force.[20] Hemorrhage from trauma is a major life-threatening condition in children. When hemorrhage coexists with thoracic injury, mortality increases to as high as 25% from 5% for hemorrhage alone. In the presence of head injuries, the mortality may reach 100%.[21-23]

Reasons behind soaring numbers of road traffic accidents

The increase in number of vehicles and expansion of road networks within and between cities and towns have contributed to the increasing number of RTAs in Saudi Arabia. Generally, traffic accidents are attributed to one of three major factors: the human factor, the vehicle, and the road/environment.

Human-related causes

Driver errors account for about 80% of all RTAs in Saudi Arabia.[16] Overspeeding is responsible for 65% of all traffic accidents (3.5 times the incidence in the USA).[1] Violation of traffic signals at urban intersections is responsible for about 50% of accidents (4.5 times more common than in the USA).[1,3] Other errors include passing another vehicle or overtaking from the wrong side (eight times more common than in the USA), driving without a license, incorrect parking, and incorrect U-turns.[1,16] Another often neglected cause is the poor maintenance of vehicles and tires. Although 90% of the drivers have claimed that they check their tires at least once a month, it was found that most of them did it incorrectly.[17] Furthermore, only 23% of the drivers knew the correct tire pressure recommended by the manufacturer for their vehicles.[17] Use of cellphones while driving is a deadly mixture, especially distracts the driver's attention from driving.[24] Another issue of major significance is noncompliance with traffic laws, especially the wearing of seat belts. Although the "wearing seat belt" law was made compulsory in Saudi Arabia on December 5, 2000, only 4% of lower socio-economic-class passengers and about 40% of middle-class passengers were found to abide by this law.[2] Most traffic fatalities are due to severe head injuries that are mostly related to the non-use of seat belts.[25] About 40% of RTAs are attributed to non-Saudis.[16] Thus, the increasing number of expatriates from different countries who are unfamiliar with local driving conditions and requirements is also an important factor in the increasing number of RTAs in the country.[1] Stress and exhaustion due to long working hours, moving to larger cities, poor education, young age, and aggressive personality traits were also found to be associated with a greater number of accidents.[6] Other general factors related to people include poor child restraint and unsupervised children playing in the streets, resulting in injuries as pedestrians.[26] Among other important contributing factors are unfamiliarity with vehicles, thrill-seeking, and overconfidence.[14] In Saudi Arabia, there is an obvious lack of a good attitude and common courtesy among some drivers who believe that they always have the right of way and every one has to yield to them. For example, instead of lining up at a traffic light, they move in front of other cars and position themselves close as possible to the traffic light. This unacceptable practice is more common among the affluent and young drivers.

Vehicle, road and environment-related causes

Vehicles and road layouts contribute to accidents and account for 20% of RTAs in Saudi Arabia.[16] People in small and light vehicles are more likely to have serious injuries and fatalities.[17] Improving vehicle manufacturing should result in less fatal road traffic injuries.[14] Tire blowouts and poor roads are very "hot" safety issues in Saudi Arabia, particularly in rural areas. The total number of tire-related accidents occurs at a rate of one accident per 11 km of rural roads according to a 2001 report on traffic accidents.[17] Another problem is that some four-lane roads suddenly become three-lane roads after an intersection or lane-marking deviations. Drivers have a hard time adjusting to this, especially if the light at the intersection is green and there is no time to adjust. Traffic signals are lacking in some smaller roads, and right-of-way is unclear. Ongoing highway construction at entrances and exits from and into service roads is another problem. Collisions are common when highway entrances and exits are in close proximity (half a kilometer) and there is little room to maneuver.

At peak times, vehicles are not allowed to enter highways at some entrances, resulting in more congestion in service roads, which defeats the purpose of having highways, which are supposed to reduce congestion. Among solutions to minimize congestion at peak times are the use of one or two lanes from the opposite side of the free highway. Environmental factors such as rain, fog, and dust have minimal effects on RTAs in Saudi Arabia.[4] However, extreme heat is responsible for 39% of all accidents due to tire blowouts.[27] Heat also contributes to driver stress levels, leading to reduced mental capacity.[28]

Ambulance services

In Riyadh, there are 7 ambulance stations with only 14 ambulances and fewer than 30 emergency medical technicians (EMTs) to cover an area of 4 million people.[29] This is not enough to serve such a highly populated urban area. In South Carolina, for example, there are 478 ambulances with 2542 active state-certified EMTs to cover an area of 3.1 million people.[29] Most of the emergencies in Saudi Arabia are transported either by volunteers or police vehicles, not by the Saudi Red Crescent (SRC) ambulances. There is a lack of reliance on the SRC that may be due to a lack of confidence in the service or a lack of awareness of its role. According to an SRC survey (Magazine of SRC Society, 1998), only 3% of those interviewed had recognized that the emergency phone number for SRC is 997, 70% had answered that it was 911, which they knew from the famous 911 US television program.[29] Although an air medical evacuation system is available between cities in Saudi Arabia, this service is limited to some seriously ill patients but is not enough to cover major injuries. In addition, getting the service is a complex process that is also relatively time-consuming and hence, a structured helicopter emergency rescue service is under study. This service is more important for the rapid evacuation of seriously injured victims to appropriate trauma facilities. In Germany, for example, helicopter evacuation of seriously injured patients to a trauma care facility is usually carried out within 15 minutes.[30]

The need for improved trauma care

The need for improved trauma care is obvious. Statistically, trauma is ranked as the number one killer in Saudi Arabia;[1] RTAs accounting for 80% to 85% of these traumas.[15] In 1986, death due to RTAs was found to be 26.5 per 10 0000 of the resident population.[13] In 2005, the number of RTAs in Saudi Arabia were 283 684 and resulted in 5883 deaths.[16] Countrywide prehospital care is suboptimal with the exception of some centers in big cities and on major roads. Rehabilitation services are also deficient, except for a few centers. Prevention, communication, transportation, hospital care, training, and public education need further emphasis.

What is the solution?

After discussing the magnitude of the problem of

RTAs in Saudi Arabia, it is obvious that the care of injured people in this country is not up to the desired standards. There have been calls for urgent and aggressive interventions to save lives and resources. RTAs involve many factors that need to be addressed collectively by all facilities that are concerned with this escalating problem. Research[8,9,10,31,32] and reports[33,34] in some countries have shown that implementation of trauma systems can significantly decrease mortality and morbidity and even the cost[35] due to trauma based on the system's efficacy. For these reasons, the development and implementation of trauma care systems in Saudi Arabia have been deemed necessary in order to improve trauma care and outcome. Sectors and institutions concerned with trauma and related problems should work together to study and discuss the feasibility of developing and implementing such a system.

What is a trauma system?

A trauma system is a planned, comprehensive, and coordinated region- or countrywide injury response network that includes all facilities and sectors with the capability to care for the injured, and is integrated with the local public health care system. This system involves components related to trauma prevention, prehospital care, hospital care, rehabilitation of the disabled, disaster medical planning, systems administration, trauma care education and training, trauma care evaluation and total quality improvement, and includes the participation of society. A country or a region should set their own goals according to the resources and facilities available. Ideally, a successful trauma system should involve the following goals:[34]

- To decrease the incidence and severity of trauma
- To ensure optimal, equitable, and accessible care for all persons sustaining trauma
- To prevent unnecessary deaths and disabilities from trauma
- To contain costs while enhancing efficiency
- To implement quality and performance improvements in trauma care throughout the system
- To ensure that certain designated facilities have appropriate resources to meet the needs of the injured

These goals can be achieved collectively at once or gradually in steps depending on the local resources. The core functions and essential services of the trauma system are summarized in Figure 1.

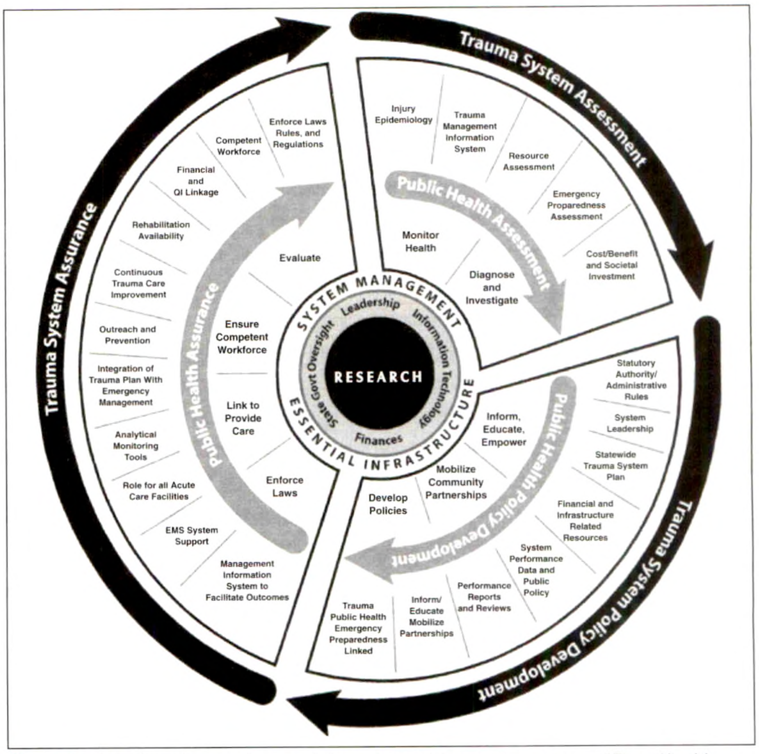
Trauma System Assessment
Trauma System Policy Development
Trauma System Assurance
Public Health Assessment
Public Health Policy Development
Public Health Assurance
RESEARCH
SYSTEM MANAGEMENT
ESSENTIAL INFRASTRUCTURE
Leadership
Information Technology
Finances
State Govt Oversight
Injury Epidemiology
Trauma Management Information System
Resource Assessment
Emergency Preparedness Assessment
Cost/Benefit and Societal Investment
Monitor Health
Diagnose and Investigate
Statutory Authority/ Administrative Rules
System Leadership
Statewide Trauma System Plan
Financial and Infrastructure Related Resources
System Performance Data and Public Policy
Performance Reports and Reviews
Inform/ Educate Mobilize Partnerships
Trauma Public Health Emergency Preparedness Linked
Inform, Educate, Empower
Mobilize Community Partnerships
Develop Policies
Enforce Laws
Link to Provide Care
Ensure Competent Workforce
Evaluate
Enforce Laws Rules, and Regulations
Competent Workforce
Financial and QI Linkage
Rehabilitation Availability
Continuous Trauma Care Improvement
Outreach and Prevention
Integration of Trauma Plan With Emergency Management
Analytical Monitoring Tools
Role for all Acute Care Facilities
EMS System Support
Management Information System to Facilitate Outcomes

Figure 1. Core functions and essential services of the trauma system. *From Health Resources and Services Administration. *Model Trauma System Planning and Evaluation.* Rockville, MD: Health Resources and Services Administration; 2006:18.[34]

Essential components of a trauma system

The first component of a trauma system is the identification of risk factors leading to injury and it needs assessment through data collection and research. After establishing the need to improve trauma care, public information and education are initiated through prevention programs and the establishment of a trauma advisory committee. A regional trauma advisory committee representing all sectors involved in the care of the injured will be responsible for trauma system leadership, legislation, and planning. Prehospital care involves communication, triage and transport to a trauma care facility, medical direction, and treatment protocols. Definitive care refers to patient management in designated trauma care facilities, interfacility transfer, and rehabilitation of the injured. Human resources are provided through workforce resources and educational preparation. System evaluation and monitoring are achieved by ongoing research through a trauma registry and are based on quality improvement data.

The trauma advisory committee (lead agency)

A trauma system is best managed through a national or regional trauma advisory committee (TAC).

Trauma-concerned community sectors should be approached to introduce them to the rationale behind planning and implementation of a trauma care system and to ensure their willingness to cooperate and participate in developing it. As trauma is an ethical and legal responsibility of the medical profession,[36] the TAC should be led by a full-time or part-time trauma medical manager and medical representatives from universities, medical schools and university hospitals, the Ministry of Health, Saudi Red Crescent, the military, Security Forces, National Guard, and private hospitals involved in trauma management. Representatives of the Police and Traffic Regulation Authority, Civil Defense, Ministry of Transportation, and Ministry of Labor and Social Affairs are also important. The TAC is the lead agency that sets legislation, enforces policies and laws, plans and develops the system, assures integration and collaboration among sectors, builds coalitions and partnerships that can assist in ensuring injury prevention, and monitors system efficacy. Other important leaders in the system include prehospital trauma care directors, trauma healthcare facility directors, rehabilitation care directors, and others. Those leaders work with the lead agency to educate and train people, ensure the implementation of the system, and assist in trauma system evaluation and research.[34] The lead agency should be supported by the government, with adequate resources that include an office for the trauma system program manager, secretarial assistance, a meeting room, data entry and analysis personnel, monitoring and law enforcement personnel, epidemiologists and researchers, and others as needed.[37]

Legislation

Legislation consist of rules and regulations set by the lead agency.[34] These may include traffic laws, rules and regulations at all levels and components of the trauma care system, and methods for their implementation. The TAC may review current legislation, modify or add new legislation, and consider their feasibility as necessary to suit national and/or regional circumstances. These rules and regulations should be applied with equal enforcement so that no one is above the law, and with continuous monitoring and periodical evaluation. Legislation should also enforce the integration and collaboration of services concerned with trauma so that a comprehensive and coordinated system can be successfully implemented with some efficiency and durability.

System planning

The planning starts with the commitment of the lead agency members and corresponding sectors involved in the care of the injured.[34] Members should have the same mindset on common philosophy and future vision for the system, share the same goals and objectives, coordinate to bridge differences in opinions, and attempt to reach a consensus for all issues.[37] Lead agency members should have a diversity of skills and experience in management, education, and clinical practice. A plan should be produced by the lead agency and other concerned agencies based on the assessment of its needs, expected challenges, known gaps and barriers, and ongoing updates. The plan should be integrated and inclusive of prevention programs, policies and protocols, operational components, facilities and resources, communications, transportation, prehospital care, hospital care facilities, and rehabilitation.

System finance

System support is a complex process that requires government and private funding, community shares, and the judicious use of available resources.[34] The government is responsible for the provision of quality trauma care to all seriously injured individuals starting from prevention, prehospital care, hospital care, and rehabilitation. Funding can be facilitated further by developing a funding framework by the lead agency and the corporation. Private companies supplying materials and equipment for system operation are also obliged to share in funding the lead agency, educational and training programs, and other activities as necessary. Various government agencies and the private sector players concerned with the care of the injured should develop their own funding plans in coordination with the lead agency and other trauma system leaders.

An operational budget plan for each component in the trauma system should be developed to adequately fund the trauma system's administrative and program priorities. Trauma system expenses must be linked ultimately to a description of the cost-effectiveness of trauma care and its demonstrated benefit to society, such as the numbers of lives saved and of injured persons returned to maximum preinjury productivity. This information will assist decision makers and the public in understanding the relationship between trauma system costs and the system's value to society and will generate support for sustained trauma system funding that may include health insurance, a surcharge of all vehicle registration, licenses, and violation penalties.

Costs of the trauma system are usually estimated through the three phases of care: prehospital, hospital, and rehabilitation.[36] Prehospital costs can be estimated by calculating the total number of injuries per year, the injury being minor or major, and the average cost per case. Hospital costs also depend on the number and category of the hospitals, such as level I, II, III, or IV. Hospital costs are estimated based on average trauma care costs per individual per year in the emergency department, intensive care unit, intermediate care unit, and regular wards. The average hospital stay for a trauma patient is about 15 days. Added to that are other cost estimations per year, including tests, surgeries, and other ancillary services. Rehabilitation costs can be estimated based on average costs per individual per number of cases per year. Staffing costs may be divided into management, clinical, and training. Management cost estimations include the annual salary for trauma directors, nurse coordinators, and clerical/office expenses. Clinical staffing costs depend on the volume of trauma patients received and the demand per hospital category. Recruitment and training costs are also important to consider in any trauma system.

Injury prevention

Injury prevention is the most important aspect of trauma system development.[33] More than half of trauma deaths occur within minutes from injury due to major organ disruptions that are not salvageable even with the best acute trauma care facilities. This portion of the injured population is best addressed by injury prevention.[38] Preventive efforts should focus on identified local risk factors and high-risk population groups. Strategies that can alter personal behavior through education and legislation have the greatest impact on trauma prevention.[39] Injury prevention efforts can be categorized into three phases:[40] primary (precrash phase), e.g., trauma prevention education and law enforcement; secondary (crash phase), e.g., wearing a seat belt; tertiary (postcrash phase), e.g., providing injured victims with optimal prehospital, hospital, and rehabilitative care. These phases involve factors related to human beings themselves, vehicles and equipment, and the environment (Table 1).

Prehospital care

Prehospital care involves prompt communication and activation of the system, proper actions at the scene of the crash by bystanders, and the prompt response of the system. This includes all the appropriate personnel, assessment, and treatment of the injured people at the scene, and transport to trauma care facilities while delivering the necessary medical care before arrival at the hospital. Training is the most important aspect of successful prehospital care. In many communities, prehospital care is provided by "basic first-aid providers" or "Emergency Medical Technicians-Basic" (EMT-Basic). Some centers have intermediate prehospital trauma care providers (EMT-Intermediate) who are able to administer intravenous therapy and apply pneumatic antishock garments in addition to basic maneuvers. A few centers have advanced prehospital training known as EMT-Advanced. Advanced EMTs are able to do endotracheal intubation, needle thoracostomy, and cricothyroidostomy.[41] The Prehospital Trauma Life Support (PHTLS) program provides basic and advanced prehospital training.[42] In addition to the importance of prehospital care provision at the scene, prompt and proper transportation to appropriate trauma care facilities are also important for better survival and outcome.[41] The "golden hour" is an approximate time frame concept emphasizing the urgency necessary for successful management of the injured patient from the time of accident to the receipt of definitive care at the hospital. It is an opportunity window for prehospital and

Table 1. Injury prevention (The Haddon matrix).[38]

Phase		Factors		
		Human	Vehicles and equipment	Environment
Precrash	Crash prevention	Information Attitudes Impairment Police enforcement	Road worthiness Lighting Braking Handling Speed management	Road design and road layout Speed limits Pedestrian facilities
Crash	Injury prevention during the crash	Use of restraints Impairment	Occupant restraints Other safety devices Crash protective design	Crash-protective roadside objects
Postcrash	Life sustaining	First-aid skill Access to medics	Ease of access Fire risk	Rescue facilities Congestion

hospital care providers to coordinate medical care with decreased impact from mortality and morbidity due to life-threatening airway, breathing, and circulatory injuries.[43,44] EMT-basic and PHTLS programs are available in Saudi Arabia; however, trained personnel are not up the desired standards for local and national needs.

Definitive care and rehabilitation

Quality definitive trauma care is the hallmark of trauma systems. Reported improvements in mortality and morbidity from trauma were mostly attributed to designated trauma centers.[32,45] Trauma centers should also participate in the essential activities of a trauma system, including prevention, performance improvement, data submission to trauma registries, representation in the trauma advisory committee, and mutual operational agreement with other regional hospitals to address interfacility transfer, educational support, and outreach.[37] In most trauma systems, a combination of levels of designated trauma centers will coexist.[46] A level I trauma center is a sophisticated definitive care facility central to the trauma system, where all severe and complex injuries are managed by an inhouse attending surgeon, other medical and administrative personnel, and ancillary services around the clock. The level I trauma center is a leader of state-of-the-art trauma care that includes quality improvement, education, and research activities. A level II trauma center is also expected to manage severe injuries with the availability of an attending surgeon and other medical personnel; however, graduate education and research are not required. Level III trauma care facilities are usually large community hospitals that can survey, resuscitate, and stabilize all trauma victims even if they need definitive surgery; however, complex cases beyond the available resources are transferred to higher centers with transfer agreements and protocols. Level IV is usually a rural trauma care facility where injured victims can receive initial assessment and management until they become stable enough for safe transfer to higher centers. In an inclusive trauma system, injured patients should be triaged and transported to an appropriate trauma care facility, not necessarily to the nearest health care facility, but to one based on injury severity. Rehabilitation of residual disability from severe injuries is an integral component of the trauma system. The goals of rehabilitative interventions are: to resume normal intellectual and physical functions; to reduce disabilities; and to avoid handicap as far as possible. Ideally, rehabilitation should be planned to start as early as possible from admission time, continue during the acute phase of care, and end if necessary in the chronic rehabilitation center. Rehabilitation representation in the trauma advisory committee is essential for the assessment of needs and national or regional distribution of rehabilitative care, provision of personnel and rehabilitation specialists, resources provision and allocation, interfacility transfer agreements, and research.[37]

Human resources (education and training)

Human resources investment involves the recruitment and retention of qualified trauma care personnel within all components of the trauma system. Specific educational and training requirements should be delineated through administrative policies and procedures based on national or regional needs of the system. These requirements are usually achieved in collaboration with national or international academic institutions and universities. Varied trauma programs that address almost all aspects of trauma management for physicians, nurses, paramedics, and other personnel are available nationally and internationally.[47] The periodic review of both the required and supplemental educational opportunities is an activity for the trauma-specific national multidisciplinary, multi-agency advisory committee.[34] There is a growing concern that patient access to trauma care facilities is becoming more difficult and that interest in trauma training and specialization is declining.[48] Acute care surgery that deals with all surgical emergencies may help in addressing the shortage of trauma surgeons.[49]

System evaluation and quality improvement

An effective system evaluation addresses two aspects: patient care and social components.[34] Patient care includes operational and clinical components in prehospital, hospital, and rehabilitation environments. Social components include legislation, prevention programs, education, research, economics, and an assessment of value of quality in relation to cost. The management information system (MIS) is a comprehensive data resource derived from various components of the trauma system, including trauma registries, prehospital registry, incident after-action reports, injury registry, death certificates, hospital administrative data sets, medical examiner's reports, crash reports, and other data. The MIS is reviewed and studied by the trauma advisory committee and other subcommittees within the system with the aim of driving public policy; enhancing system performance; providing guidance for injury prevention activities and education of trauma care providers;

identifying and evaluating system best practices; identifying and evaluating gaps in the system; reassessing trauma resource utilization; tracking patient outcomes; developing performance standards; and measuring system performance against similar systems. The quality of medical care has evolved over the years from simple medical audits, through performance improvement and quality assurance, to quality improvement (QI).[47] The same thing can be applied to trauma QI, which involves mortality and morbidity meetings, preventable death studies, audit filters, complications, and unexpected (risk adjusted) mortality. For the methods noted above, the primary principle is to identify problems that arise due to correctable factors.[50,51] Corrective actions should be taken to improve these problems and the effects of these actions are evaluated to assess whether they have been successful in correcting the problem. Other methods of trauma QI involve prehospital care reviews, hospital inspection, and continuing education. The QI program is an integral part of the trauma system that contributes to improvement in the trauma care process, decreases mortality, and decreases costs.[10]

Research

Research is the core of trauma care systems.[34] It drives the system and provides the foundation for system development and performance improvement.[37] Research data from all components of the system should be reliable and made available to all investigators. Competitive grants or contracts made available through lead authorities or constituencies should provide funds to support research activities. The trauma registry[52] provides investigators with information about patient demographics, prevalence and incidence of injuries, other injury details, patterns of care provided within the system, and outcomes. The lead agency is responsible for administration, confidentiality, security, and access to trauma registries. Other population-based data that are not usually entered into trauma registries (such as deaths at the scene, patients managed at nontrauma designated hospitals, or patients with injuries that are not severe enough to require admission) are also important to capture and document for comprehensive system development and research. Multidisciplinary participation in research is essential for the reliability and validity of the research work, which reflects all components of the system, and not only the medical care. Investigators from various disciplines should be selected carefully by the lead agency based on their qualifications and expertise in research in collaboration with academic centers and public health agencies. Analyzed data, conclusions, recommendations, and alterations in the system should be published in peer-reviewed journals and communicated to the public through presentations, annual reviews, and public information formats.

Conclusion

Trauma in Saudi Arabia is a major public health problem with increasing rates of mortality and morbidity. The socioeconomic burden, depletion of human resources, the emotional and psychological stress on families, and the strain on healthcare facilities are also increasing. To minimize this impact, a national multidisciplinary trauma system has to be developed and implemented before it is too late to manage the further complexities of trauma in the future.

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brief report

Characteristics of pandemic influenza A (H1N1) infection in patients presenting to a university hospital in Riyadh, Saudi Arabia

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BACKGROUND AND OBJECTIVES: A national plan of management for flu-like illnesses was developed by the Saudi Ministry of Health after the first outbreak in Saudi Arabia in June. We describe the clinical presentation of the H1N1 cases attending King Khalid University Hospital (KKUH) between July through September 2009 and identify the high-risk age groups.

METHODS: All patients presenting with influenza-like illnesses (ILI) in the H1N1 clinics during the specified period were clinically examined and tested using reverse transcription polymerase chain reaction (RT-PCR). Those who were clinically diagnosed and confirmed positive for novel influenza A (H1N1) were included in the study.

RESULTS: Over a 6-week period, 117 cases of laboratory-confirmed cases were reported in KKUH with a mean (SD) age of 19.6 (16.7) years, of whom 72 (62.1%) were males. Most reported cases were Saudis (n=99, 85.3%); 94 (81%) had no travel history outside the country; 100 (86.2%) had had no contact with an H1N1-identified patient; 33% were aged 5-14 years and 28.4% were aged 15-29 years. The most commonly reported symptoms were fever in 99 (85.3%), cough in 9 (81%), runny nose (33.6%) and sore throat (21.3%). All 117 cases were confirmed positive using real time RT-PCR testing. Thirty-one cases (26%) were admitted and 22 of those (71%) recovered after receiving oseltamivir. Two deaths were attributed to the 2009 pandemic. One patient died of chronic pulmonary disease. The other cause of death was unknown.

CONCLUSION: These findings indicate indigenous influenza A (H1N1) transmission, and confirm the urgent need for prevention strategies which specifically target children and young adults, who appear to have a higher risk of infection and hospitalization. Such measures include immunization, improved personal hygiene, and increased ventilation in habitations.

The World Health Organization (WHO) Global Influenza Surveillance Network (GISN) continues to monitor the global circulation of influenza viruses and seasonal viruses. From the beginning of the declared pandemic on April 19 to September 12, 2009 a total of 80 countries had reported cases. The total number of specimens reported positive for influenza viruses by WHO-designated National Influenza Centers laboratories was 110 969. Of these, 67 207 (60.6%) were pandemic H1N1 and 6054 (5.5%) were seasonal A (H1). Data from issued reports issued for the week September 6 to September 12, 2009 (a total of 20 countries) show that the total number of specimens reporting positive for influenza viruses by NIC laboratories was 5862. Of these, 4444 (75.8 %) were pandemic H1N1 and 155 (2.6 %) were seasonal A (H1). On average, the pandemic A (H1N1) accounted for 76% of all subtypes of influenza detected (76% in the northern hemisphere and 87% in the southern hemisphere).[1]

On June 3, 2009 the first case of pandemic influenza A (H1N1) virus was reported in Saudi Arabia.[2] On September 12, 2009, the Saudi Ministry of Health issued a national plan of management for flu-like pandemics, specifically pandemic influenza A (H1N1) virus infections.[3] Moreover, the Saudi Ministry of Interior published an action plan to manage the mass influx of travelers arriving for the Islamic pilgrimages of Ummrah and Hajj.[4] The action plan recommended obligatory pre-travel vaccination against seasonal and/or H1N1 influenza, an awareness campaign for preventive procedures, a compulsory health assessment and follow-up, and for their own safety, urged high-risk groups (elderly, patients with chronic diseases, children and pregnant women) to avoid travelling. This report summarizes laboratory-confirmed cases identified during the period from July 1 to September 12, 2009 at King Khalid University Hospital, Riyadh, Saudi Arabia.

METHODS

The data was collected using a specially designed form based upon KKUH medical records covering a period of 6 weeks (July 1 to September 12, 2009). A total of 117 subjects, identified through H1N1 clinics were diagnosed for influenza-like illness on a clinical basis and confirmed positive for novel influenza A (H1N1) through RT-PCR testing (Roche, Germany). Subjects who reported having an ILI (defined as an oral temperature of more than 38°C (100.4°F) or a history of fever or chills and at least one influenza-like symptom) were asked to provide specimens for virologic testing via nasal and throat swabs. The diagnostic test was a real-time RT-PCR assay that uses fluorogenic hydrolysis probe technology for the detection of human influenza A viruses, and the differential detection of 2009 H1N1 influenza virus in nasopharyngeal swabs (NPS), nasal swabs (NS), throat swabs (TS), and nasal aspirates (NA) according to the manufacturer's instructions (Roche, Germany) using specific probes for the novel influenza A (H1N1) strain.

RESULTS

By September 12, 2009 a total of 117 laboratory confirmed cases were reported at KKUH. Of these, 72 (62.1%) were males and 42 (36.2%) females. Gender information was missing in three cases. The mean age was 19.6 (16.7) years. The age of cases ranged from 2 months to 75 years. Nevertheless, most reported cases were in the age range of 5 to 14 years followed by 15 to 29 years (Table 1). The fewest cases were reported for children aged 0 to 4 years. The majority of the patients (n=99, 85.3%) were Saudis. The remainder included 7 Filipinos, 1 Palestinian and 1 Pakistani. Nationality information was missing in nine cases. Most of these patients (n=94, 81%) had not travelled out of the country, while 14 had travelled within the Middle East, 6 to Europe and only 2 to Asia. Most interestingly, an unexpectedly large number of cases confirmed that they had not had contact with an H1N1-identified patient (n=100, 86.2%) nor any contact with a patient with any symptoms of influenza (n=83, 71.6%).

Table 1. Laboratory-confirmed H1N1 cases reported to King Khalid University Hospital, July 1 –September 12, 2009, by age.

Age groups in years	No. of H1N1 cases	Percent
0-4	18	16.5
5-14	36	33.0
15-29	31	28.4
30-59	21	19.3
≥60	3	2.8
Total	**109**	**100**

Information missing in 8 patients

Among 116 patients for whom the data for signs and symptoms were available, the most commonly reported symptoms are shown in Table 2. The most frequently reported groups of symptoms were: fever and cough (n=30, 25.9%); fever, cough and sore throat (n=19 patients, 16.4%); and fever, cough and runny nose (n=13, 11.2 %).

Figure 1 shows the number of cases that were confirmed positive using the Rapid Test (Becton-Dickinson, USA) and the polymerase chain reaction (PCR) test over 12 weeks from July 1 to September 12, 2009. Of the total number of cases, only 25 were tested for H1N1, with the Rapid Test showing 15 cases as positive (11 reported in the month of August and 4 in September), while the remaining 10 cases were reported negative. As per the selection criteria, all 117 cases were confirmed positive using the PCR test. According to the results, 15 cases were reported in July, 55 in August and 45 in September. Two cases had missing data on the date of diagnosis. Prior to July 15, 2009, KKUH referred the screening Rapid Test and PCR for H1N1 to external diagnostic laboratories. The start date of testing within KKUH laboratories is marked on Figure 1. For the data recorded as of week 12 (mid-week of September 2009), most cases, (n=85, 73%) were diagnosed in the outpatient clinic and sent home with specific medical advice. About one-third (31 cases) were admitted as in-patients, out of whom 22 recovered after receiving a treatment regimen of oseltamivir phosphate (Tamiflu, Roche) capsules for adults and a suspension for children in a dose of 75 mg twice per day for five days. All patients were subsequently discharged. Nine inpatients were still undergoing treatment when this report was prepared. The data also showed that out of those admitted, 12 cases suffered from co-morbidities (2 with pulmonary disease; 1 with cardiac disease; 2 with chronic renal/liver diseases; 1 with diabetes; 1 was overweight as determined by BMI; and 5 were suffering other diseases). Additionally, there was one pregnant woman and a lactating mother with a reported decreased milk volume. As of September 12, 2009 there were two reported deaths of west Riyadh residents attributed to the 2009 pandemic influenza A (H1N1) virus infection; one had chronic pulmonary disease and the other, from outside the KKUH, had unknown contributory diseases.[5]

DISCUSSION

To raise awareness about the status of the novel influenza A (H1N1) and prevention and control efforts, the King Saud University (KSU) of Riyadh established the Standing Epidemic Control Committee (SECC). The SECC has sent several health educational alerts to KSU employees and students, as well as the nearby general community through the University media and electronic network. It is thought that these messages contributed to the successful early identification of cases. Thus, as of September 12, 2009 the 117 cases reported to KKUH (one of about 100 public and private hospitals in Riyadh) constituted 6% of the overall nationally reported confirmed cases at that time. This reflects the high awareness of KKUH health-care providers (including different health professionals) of the outbreak, leading to high public awareness, which could have enhanced hospital laboratory surveillance activities.

During the 6-week period covered by this report, the 117 confirmed cases of 2009 pandemic influenza A (H1N1) virus infection were reported to the Infection Control Unit at KKUH by the H1N1 university clinics. The highest proportion of cases, both overall and among hospitalized patients, were among children aged 5 to 14 years or adolescent/young adults aged 15 to 29 years (>60%), with a substantially lower proportion aged ≥60 years. Recent reports from Mexico and USA[6,7] indicating that age-specific attack rates for the 2009 pandemic Influenza A (H1N1) virus infection cases are higher in younger persons and lower in older persons (compared with seasonal influenza infections) concur with the results of this study. Further explanations are provided by two Center for Disease Control (CDC, US) reports, which suggest that older persons,

Table 2. Laboratory -confirmed H1N1 cases reported to King Khalid University Hospital, July 1 to September 12, 2009, by signs and symptoms.

Multiple symptoms reported	No. of H1N1 cases	Percentage (n=117)
Adult symptoms		
Shortness of breath	17	14.7
Fever for more than 3 days while being medicated	7	6
Chest pain	6	5.2
Hemoptysis	3	2.6
Cyanosis	2	1.7
Pediatric symptoms		
Shortness of breath and tachypnea	19	16.4
Disinterest in playing	3	2.6
Common signs and symptoms		
Fever	99	85.3
Cough	94	81
Running nose	39	33.6
Sore throat	25	21.3
Vomiting	11	9.5
Body pain	10	8.5
Headache	8	6.8
Unspecified	6	5.1
Rigor	5	4.3
Diarrhea	4	3.4
Chills	3	2.6
Eyelid puffiness	2	1.7
Nausea	2	1.7
Epigastic pain	1	0.8
Body weakness	1	0.8
Fatigue	1	0.8
Abdominal pain	1	0.8
Decreased feeding	1	0.8

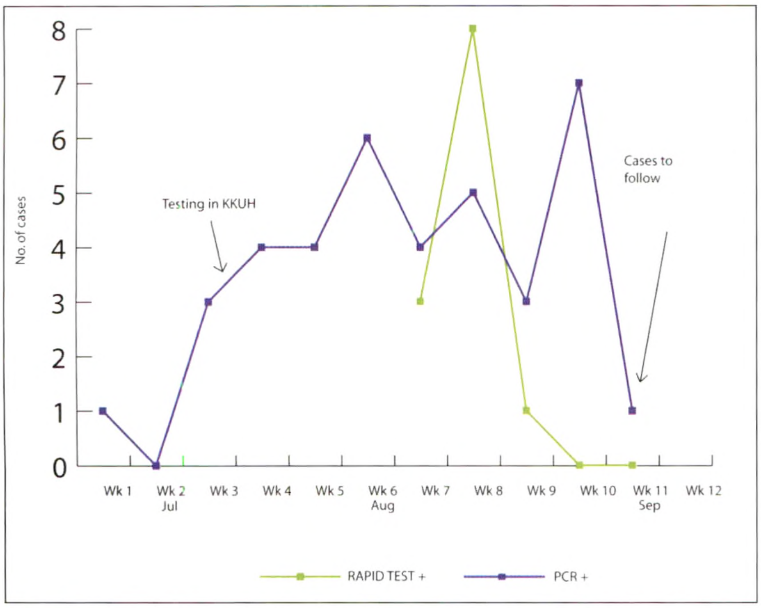

Figure 1. Laboratory-confirmed cases of H1N1 reported to King Khalid University Hospital, July 1 to September 12, 2009.

as a group, may have pre-existing immunity to the 2009 H1N1 virus[8] or a cross-reactive antibody to it, compared with none detected among children.[9] Another factor which may contribute to higher rates among children/adolescents could be greater contact rates among teenagers, as reported by a recent Japanese study.[10] Such an age differential could be also be attributed to a variation in exposure and susceptibility in accordance with the transition from a younger to older age.

The great majority of affected patients reported that they had not travelled outside of the country (81 %), had not had contact with any H1N1-identified patient (86.2%) and had not had contact with a patient with symptoms of influenza (71.6%). Such findings suggest an indigenous transmission of the novel influenza A (H1N1) virus within the country, which may have significant implications in rapid spread of the infection during mass gatherings. These findings also confirm the urgent need for prevention strategies that specifically target children and young adults who are at a higher risk of infection and hospitalization. The national plan for management of influenza pandemics and the CDC Advisory Committee on Immunization Practices (ACIP)[11] recommend that these population sub-groups should be among the first groups targeted for vaccination with the influenza A (H1N1) 2009 monovalent vaccine, once available and locally approved. The WHO mentions that the length of the approval process depends on factors such as each country's regulatory pathway, the type of vaccine being licensed, and the stage of manufacturers' readiness to submit appropriate information to regulatory authorities.[5] The finding of three elderly cases above the age of 60 years in this study series negates a common medical myth in Saudi Arabia that a majority of the high-risk population has been vaccinated. Thus, it is recommended that the campaign of vaccination for high-risk groups in the population be mobilized efficiently. Moreover, the data on pregnant and lactating mothers serves as a potential empirical gap[12] especially in a cultural context and implies the need for specific attention.

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brief report

Appendiceal ascariasis in children

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BACKGROUND: The propensity of *Ascaris lumbricoides* to wander leads to varied surgical complications in the abdomen. Wandering *A lumbricoides* may sometimes reach the vermiform appendix and its presence there may remain silent or incite pathology. Our aim was to study ascariadial appendicitis.

METHODS: Over a period of 3 years, we identified children who were found to have appendiceal ascariasis during surgery for different intestinal complications due to ascariasis. We studied the relationship between ascariasis and its lodgement inside the vermiform appendix in these patients. No preoperative diagnosis was made in this series.

RESULTS: We found 11 patients with appendiceal ascariasis. It was incidentally found that 8/11 (72.7%) patients had worms inside their vermiform appendix but not appendicitis, whereas the remaining three patients (27.2%) were found to have *Ascaris*-associated appendicitis. The characteristic finding in *Ascaris*-infested vermiform appendix was that the worm is positioned with its head at the base and its tail at the tip of the appendix.

CONCLUSION: Migration of *A lumbrocoides* inside the vermiform appendix is an incidental finding and tends to pursue a silent course in most patients. Only rarely does the presence of *Ascaris* inside the vermiform appendix cause appendicitis.

Ascaris lumbricoides is rarely seen in the vermiform appendix although they are seen in the intestines of individuals in tropical countries. *Ascaris*-associated appendicitis is a form of wandering ascariasis and is usually the sequelae of a high intestinal worm load.[1,2] *Ascaris* can be found in the normal appendix but may also be associated with appendicitis. We studied the clinical and pathological sequelae of the migration of *Ascaris* to the appendix.

METHODS

Between May 2005 and May 2008, we identified children who were found to have appendiceal ascariasis during surgery for different intestinal complications due to ascariasis at SMHS Hospital. Age, sex, clinical features, operative diagnosis, and pathological findings confirmed by histological examination were recorded for patients whose vermiform appendix showed the presence of *Ascaris*.

RESULTS

During a three-year period, a total of 11 patients with appendiceal ascariasis were encountered. Ten (91%) of these 11 patients were male and one was female (9%) (Table 1). Worms were found in the appendix during the course of surgery for ascaridial intestinal obstruction. Intestinal obstruction by worms was observed in seven patients (63.6%), adhesion obstruction (not related to *Ascaris*) in one patient (9%), appendicitis in two patients (18%), and peritonitis in one patient (9%).

Worms were found incidentally in the appendix of five patients (45.4%) who had kneading of worms, in two patients (18%) who had enterotomy for worms (Figures 1-4), during appendectomy for appendicitis for two patients (18%) (Figures 5 and 6), during the course of incidental appendectomy in one patient (9%) who had adhesional intestinal obstruction, and in one patient (9%) who had appendectomy for perforated appendix. The number of worms in the appendix ranged from one to three worms. A single worm was found in the

Table 1. Patient characteristics and pathology of appendix.

Patient number	Age (years)	Sex	Symptoms	Preoperative diagnosis	Number of worms in the appendix	Treatment	Appendicitis
1	4	F	Abdominal pain, fever, vomiting, worms in the stool	Peritonitis	(5 worms in the peritoneal cavity)	Appendectomy	Yes
2	4	M	Abdominal pain, fever, vomiting of worms, constipation	Intestinal obstruction by worms	1	Enterotomy, appendectomy	No
3	4	M	Abdominal pain, vomiting of worms, worms in the stool	Intestinal obstruction by worms	1	Kneading, appendectomy	No
4	5	M	Abdominal pain, constipation	Intestinal obstruction by worms	1	Kneading, appendectomy	No
5	5	M	Abdominal pain, vomiting of worms, worms in the stool, diarrhea	Intestinal obstruction by worms	3	Kneading, appendectomy	No
6	6	M	Abdominal pain, fever, vomiting of worms, constipation	Intestinal obstruction by worms	1	Enterotomy, appendectomy	No
7	7	M	Abdominal pain, constipation	Intestinal obstruction by worms	1	Kneading, appendectomy	No
8	8	M	Right lower abdominal pain, fever, diarrhea	Appendicitis	1	Appendectomy	Yes
9	8	M	Abdominal pain, vomiting, constipation	Intestinal obstruction by worms	1	Kneading, appendectomy	No
10	11	M	Right lower abdominal pain, fever, worms in the stool	Appendicitis	1	Appendectomy	Yes
11	12	M	Abdominal pain, vomiting, constipation	Intestinal obstruction by adhesion	1	Adhesionolysis, appendectomy	No

vermiform appendix in nine patients (81.8%) and three worms were found in the vermiform appendix in one patient (9%). A characteristic finding was that worms in the appendix had their heads at the base and their tails at the tip of the appendix. One patient (9%) had five worms in the peritoneal cavity after perforation of the appendix. Only three patients (27.2%) had histopathologically documented evidence of *Ascaris*-associated appendicitis while the other eight cases were found to have normal appendix on histopathological examination (Figure 7). Laparotomy revealed worms in the appendix of three patients (27.2%) through the appendiceal wall. Live worms were observed whenever a diagnosis was made intraoperatively.

DISCUSSION

Surgical manifestations of abdominal ascariasis are varied and are attributed to the wandering nature of *Ascaris lumbricoides*. The preoperative diagnosis of this condition continues to remain difficult, although the parasite can sometimes be observed inside the lumen during micropathological examination. Appendicitis due to the migration of *Ascaris lumbrocoides* into the appendix is still debatable because the symptoms of this migration may simulate appendicitis, but rarely cause it.[3,4] The hypothesis that *Ascaris* lumbricoides is a major cause of appendicitis in children has been disproved.[5]

In *Ascaris* infestations associated with a normal appendix, *Ascaris* lodges in the appendix and comes and

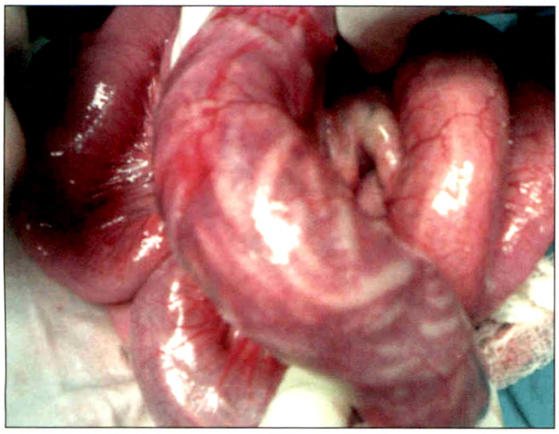

Figure 1. Long impacted worm bolus with transerosal visbility in a child who had incidental finding of worm inside appendix.

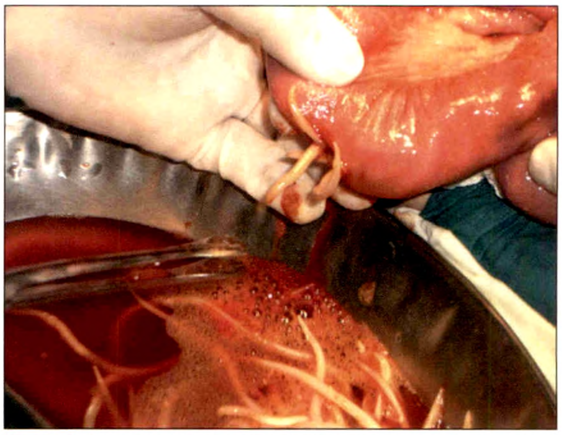

Figure 2. Enterotomy being done for impacted worm bolus in a child.

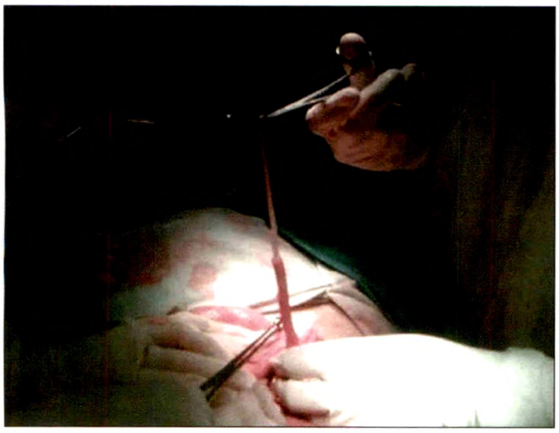

Figure 3. *Ascaris lumbricoides* being removed through tip of vermiform appendix in grossly normal appendix which has no evidence of any appendicitis at histopathology.

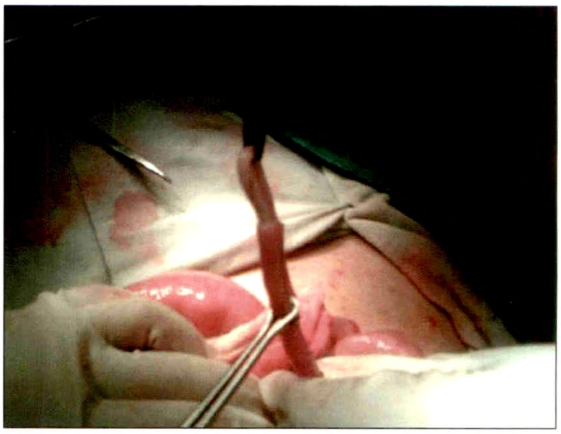

Figure 4. Second worm being removed through above normal appendix .Worm lying with tail end at tip of appendix, held at tail end and being removed.

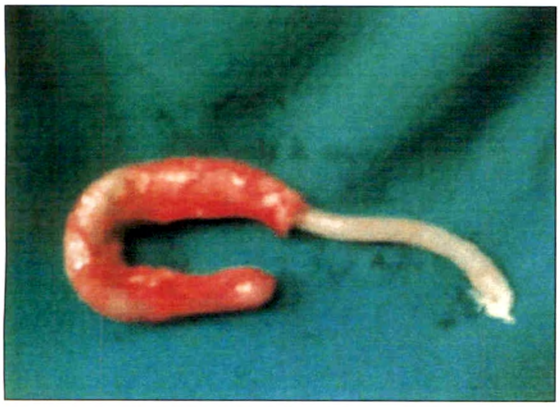

Figure 5. *Ascaris lumbricoides* with head end at base of appendix in a grossly inflammed appendix which had features of ascaridial appendicitis on histopathology.

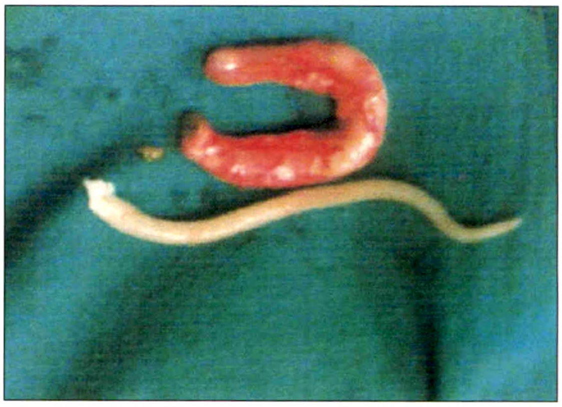

Figure 6. *Ascaris lumbrocoides* which was lying with tail end lying at the tip and head end at base of grossly inflammed vermiform appendix.

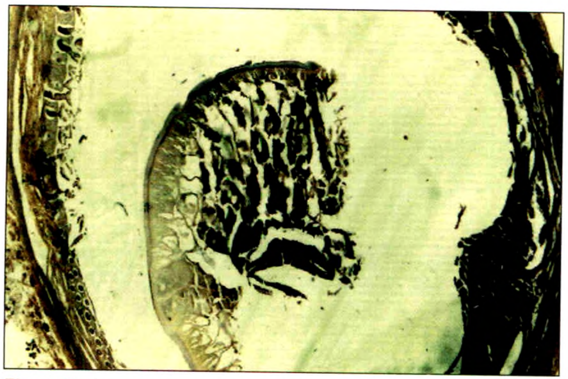

Figure 7. Cross section of vermiform appendix having *Ascaris lumbrocoides* in lumen; no features of appendicitis can be seen.

goes on its own accounting for the intermittent pain observed sometimes in children with high worm load. During the kneading of the worms, this high intestinal worm load coupled with a competent illeaocecal valve can sometimes provide a high load of worms in the cecum. This leads to the entry of the worms into the lumen of the appendix to escape the kneading. A competent ileocecal valve prevents the worms from escaping through the retrograde route. An incompetent ileocecal valve with proximal worm bolus obstruction may force the worm to travel again towards the cecum. This further contributes to the worm load in the cecum and in an attempt to seek natural orifices, the worms may enter the vermiform appendix. This type of *Ascaris*-associated normal appendix occurs in the wide-lumen, free-lying appendix. More than one worm can be seen in the lumen even when there are no grossly or microscopically visible features of appendicitis. An inflamed appendix can contain worms inside its lumen although it is debatable whether the worms caused the inflammation or whether they migrated to an already inflamed appendix. However, the presence of live worms and the associated pathology of the appendix do not favor the hypothesis that the worms cause appendicitis. Also, the presence of *Ascaris* inside the inflamed appendix favors the hypothesis that *Ascaris* has an affinity for pathological tissue. The wandering nature of *Ascaris lumbricodes* makes these worms seek openings just as they do in the perforated appendix wherein they reach the perforation site and lodge freely in the peritoneal cavity.

One of the characteristic findings of this study was that the worms were seen in the appendix with their heads at the base and their tail ends at the tip end of the appendix, which might lead to the frequent escape of worms from the appendix. *Ascaris* can be removed through the distal tip of the appendix when more than one worm is seen inside the appendix. It is to be stressed that complete removal of worms from the appendix is to be done when only a portion of the worm is lying inside the appendix and part of it is inside the cecum to avoid necrosis of the portion inside the appendicular stump, which may lead to fecal fistula. Our observations support the direct evidence of the presence of *Ascaris* in the vermiform appendix in contrast to reports of indirect evidence of migration of the worms into the appendix due to the presence of *Ascaris lumbricoides* eggs lodged in the appendix without any features of appendicitis.[6]

In conclusion, *Ascaris lumbrocoides* is rarely found in the appendix and its presence there is rarely associated with appendicitis. Worms in the appendix can be extracted through the distal tip of the appendix. *Ascaris* position themselves with their heads directed towards the base and their tail ends at the tip of the vermiform appendix.

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case report

Bleeding duodenal ulcer after Roux-en-Y gastric bypass surgery: the value of laparoscopic gastroduodenoscopy

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Roux-en-Y gastric bypass is a common surgical procedure used to treat patients with morbid obesity. One of the rare, but potentially fatal complications of gastric bypass is upper gastrointestinal bleeding, which can pose diagnostic and therapeutic dilemmas. This report describes a 39-year-old male with morbid obesity who underwent a Roux-en-Y gastric bypass. Three months postoperatively, he sustained repeated and severe upper attacks of upper gastrointestinal bleeding. He received multiple blood transfusions, and had repeated upper and lower endoscopies with no diagnostic yield. Finally, he underwent laparoscopic endoscopy which revealed a bleeding duodenal ulcer. About 5 ml of saline with adrenaline was injected, followed by electrocoagulation to seal the overlying cleft and blood vessel. He was also treated with a course of a proton pump inhibitor and given treatment for *H pylori* eradication with no further attacks of bleeding. Taking in consideration the difficulties in accessing the bypassed stomach endoscopically, laparoscopic endoscopy is a feasible and valuable diagnostic and therapeutic procedure in patients who had gastric bypass.

Postoperative upper gastrointestinal bleeding, though rare, is a potentially fatal complication following gastric bypass surgery.[1-3] One reason for this is that endoscopic access to the excluded stomach and duodenum is difficult. A variety of techniques and diagnostic methods have been suggested to visualize the excluded stomach and duodenum. These include endoscopy via percutaneous gastrostomy, retrograde endoscopy, virtual gastroscopy using CT scan, and intraoperative gastroscopy.[1-8] We present a case of a recurrent bleeding peptic ulcer following gastric bypass that was diagnosed and treated with the help of laparoscopic endoscopy.

CASE

A 39-year-old male with morbid obesity (weight=207 kg) with no other medical problems, was referred to our hospital. He underwent a Roux-en-Y gastric bypass in November 2006. Three months postoperatively, he was admitted to another hospital with melena and a drop in his hemoglobin (Hb) from 13 g/dL to 7 g/dL. He received 5 units of packed red blood cells and was started on proton pump inhibitors. He was admitted to our hospital on April 26, 2007 with similar complaints. His hemoglobin was 8.7 g/dL MCV-89.8, MCH-30.1, PLATS-258, WBC-6.1, RDW-14.3, IRON-2 umol/l. He denied any history of use of nonsteroidal anti-inflammatory drugs (NSAIDs). He was given 2 units of packed red blood cells. He underwent upper gastrointestinal endoscopy as well as colonoscopy. Upper endoscopy revealed a normal esophagus; normal remnant of the stomach, with no anastomotic ulcer; and normal jejunal loop. The colonoscopy was also normal. Biopsy from the stomach remnant showed mild chronic gastritis and was positive for *Helicobacter pylori*. He was given a course of proton pump inhibitor (Esomeprazole 20 mg twice daily for I week followed by Esomeprazol 40 mg once daily for 6 weeks) and treatment for H pylori (Amoxicillin 1000 mg twice daily and Clarithromycin 500 mg twice daily for one week). There was no further bleeding and he was discharged with a hemoglobin of 9.7 g/dl. On July 22, 2007, he was readmitted with melena. Upper gastrointestinal endoscopy did not reveal the source of the bleeding. His hemoglobin on discharge was 10.4 g/dl. On February 9, 2008 he presented to our hospital with a 3-day history of melena and his hemoglobin dropped from 10.6 g/dL to 8 g/dL. He underwent an upper gastrointestinal endoscopy, which was normal. We decided to perform laparoscopic gastroduodenoscopy to evaluate the residual stomach and duodenum.

With the patient under general anesthesia, three ports were used: a size 12-mm trocar was introduced in the upper midline, below the xiphisternum, for the camera; a size 12-mm trocar was introduced in the right upper quadrant; and a size 15-mm trocar was introduced in the left upper quadrant. The residual stomach was identified and a gastrostomy was made using diathermy scissors. The gastroscope was introduced through the 15-mm trocar in the left upper quadrant and guided into the residual stomach via the already made gastrostomy (Figure 1). This revealed old blood covering the gastric mucosa. There was also a fresh duodenal ulcer with a clot over the base (Figure 2). About 5 mL of saline with adrenaline was injected, followed by electrocoagulation to seal the overlying cleft and blood vessel. The gastrostomy was closed with endo-GIA. The trocar wounds were also closed. Postoperatively, the patient did well and was started on a course of a proton pump inhibitor and given treatment for *H pylori* eradication. Ten months postoperatively he was doing well with no complaints. His follow-up hemoglobin level was 13.3 g/dL in August 2008.

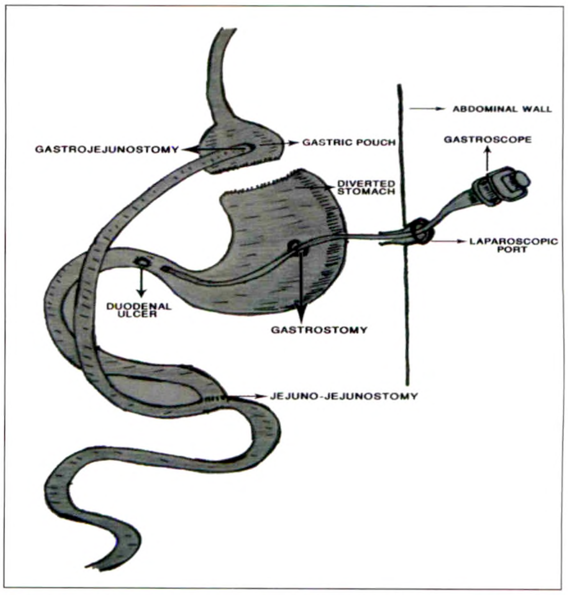

Figure 1. Diagrammatic representation of the laparoscopic endoscopy in our patient who had had Roux-en-Y gastric bypass for morbid obesity. The endoscope was introduced into the abdomen through a laparoscopic port and then into the diverted stomach through a gastrostomy incision.

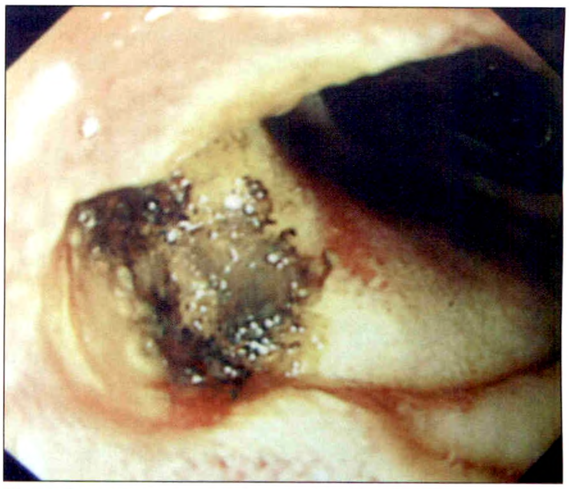

Figure 2. Intraoperative picture via the gastroscope showing the duodenal ulcer.

DISCUSSION

Morbid obesity is increasing worldwide. In the US, in 2002, 5.1% of adults were considered to be morbidly obese, having a body mass index of 40 or higher.[9] This resulted in an increase in the number of gastric bypass surgeries. In the US, 13 365 bariatric surgical procedures were performed in 1998, and this increased to 205 000 in 2007.[1,10] Gastric bypass is considered the most common bariatric surgical procedure, accounting for about 88% of all surgeries performed for obesity.[10] The increase in the number of bariatric gastric bypass procedures resulted in an increase in the number of postsurgical complications. One serious and challenging complication following Roux-en-Y gastric bypass is upper gastrointestinal bleeding. It is commonly the result of bleeding from marginal ulcers at the gastrojejunal anastomosis.[11,12] This has been reported in as many as 7% of all patients who have had Roux-en-Y gastric bypass.[11] Jamil et al, in a large series of 933 patients who underwent laparoscopic Roux-en-Y gastric bypass, reported a 3.2% incidence of postoperative upper gastrointestinal bleeding.[12] All these patients were found to have bleeding from the gastrojejunostomy staple line. The diagnosis of this complication is not difficult with the aid of esophagogastroscopy. On the other hand, a peptic ulcer in the bypassed stomach in patients who have had a Roux-en-Y gastric bypass poses both diagnostic and therapeutic difficulties. It has been shown that the bypassed gastric segment retains its ability to secrete acid and respond to vagal and hormonal stimuli.[13,14] The presence of other factors such as smoking, the use of NSAIDs, and *H pylori* infection can contribute to the development of a peptic ulcer. Our patient had *H pylori* isolated from the gastric pouch. This could have contributed to the development of the peptic ulcer in the excluded stomach. These ulcers are liable to develop complications such as bleeding and perforation.[1,3]

The diagnosis of bleeding duodenal and antral ulcers in patients who have had Roux-en-Y gastric bypass for morbid obesity is difficult. The main reason for this is the inaccessibility of the excluded stomach because of the altered anatomy. To obviate this, life-long proton pump inhibitors have been advocated for all patients who undergo Roux-en-Y gastric bypass.[15] The standard gastroscope is not long enough to reach the duodenum and stomach and therefore the use of longer endoscopes has also been suggested. Upper esophagogastroscopy is however valuable to exclude marginal ulcers at the jejunogastric anastomosis. This is also the case when using transcolonic endoscopes as they are not long enough to reach the Roux limbs. To overcome this, a variety of diagnostic and therapeutic procedures have been advocated.[1-8] For example a Tc-99m RBC scan or a celiac angiogram can be used to localize the site of the upper gastrointestinal bleeding; the latter can also be used to stop the bleeding once identified. Other methods that have been used include CT scan, CT angiogram, endoscopy via percutaneous gastrostomy, and intraoperative endoscopy. The technique we have described is simple and is less invasive than a laparotomy. It proved valuable as a diagnostic and therapeutic procedure.

In conclusion, upper gastrointestinal bleeding in patients who have had Roux-en-Y gastric bypass for morbid obesity presents unique diagnostic and therapeutic challenges, both for the bariatric surgeons and gastroenterologists, because of difficulties in accessing the bypassed stomach endoscopically. Laparoscopic endoscopy is a feasible and valuable diagnostic and therapeutic procedure in these patients.

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Combined occurrence of diabetes mellitus and retinitis pigmentosa

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The combined occurrence of diabetes mellitus (DM) and retinitis pigmentosa (RP) is rare. It has been reported in the form of four different syndromes that are inherited in an autosomal recessive fashion. We describe two cases of DM and RP occurring together. The first case was a 35-year-old male who presented with insulin-treated diabetes, obesity, hypertension, polydactyly, normal cognitive functions, an ataxic gait, blindness secondary to RP, dyslipidemia, impaired renal function, and multiple renal cysts. He was diagnosed clinically as having Bardet-Biedl syndrome. The second case was a 34-year-old male who presented with insulin-resistant diabetes, hypertension, blindness secondary to RP, deafness, normal cognitive functions, primary infertility, renal, and liver impairment. He was diagnosed clinically as having Alström syndrome. Because of overlapping clinical manifestations and the cost and time involved in genetic studies, clinical criteria can be used for diagnosis and as a guide for genetic mapping in these patients.

Although diabetes mellitus (DM) and retinitis pigmentosa (RP) are common and occur independently, the combination of both conditions is rarely seen in clinical practice. Four syndromes have been reported[2,8,15,20] describing the combination of both conditions that were coexistent with other manifestations such as obesity, deafness, hypogonadism, renal abnormalities, and/or mental retardation. The inheritance of these syndromes is autosomal recessive. Diagnosis can be done clinically based on primary and secondary features, but molecular genetic testing may be needed for accurate diagnosis and genetic counselling. In this report, we describe two cases who presented with diabetes and retinitis pigmentosa along with other manifestations. In both cases, the diagnosis was established on the basis of clinical findings. The first case was diagnosed as Bardet-Biedl syndrome based on the clinical presentation of DM with RP along with obesity, polydactyly, renal cortical cysts, and ataxic gait, whereas the second case was diagnosed as Alström syndrome based on the clinical presentation of RP and insulin-resistant diabetes, deafness, hepatic dysfunction, renal impairment, and hypogonadism. We also reviewed the literature summarizing the clinical manifestations and the mode of inheritance of each syndrome.

CASE 1

A 35 year-old Iraqi male was referred to our diabetes clinic for insulin-treated DM, impaired renal function with a serum creatinine level of 188 mmol/L (normal reference range is 45-105 mmol/L), gross proteinuria of 2.7 g/day, and a fasting blood sugar level of 13.7 mmol/L (RR is 3.9-6.1 mmol/L). The patient had been diagnosed with Laurene-Moon-Biedl syndrome since 1990, with a history of night blindness and gradual visual deterioration since childhood. He was ultimately registered as being blind in 1999, when he was also found to be diabetic. He was hypertensive and dyslipidemic; his parents were first cousins. Clinical examination revealed a weight of 120 kg, a height of 180 cm, a body mass index (BMI) of 36.3 kg/m^2, and a BP of 140/100 mm Hg. He had polydactyly of the left hand and both feet (Figure 1). Examination of the heart, abdomen, and chest did not reveal any abnormalities. Neurologically, he had normal cognitive functions, but had an ataxic gait and diminished vibration and pain sensation over both the lower limbs with intact peripheral pulsation. Fundus examination revealed retinitis pigmentosa and optic atrophy (Figure 2, Panel a). His biochemical profile showed an elevated triglyceride level at 3.54 mmol/L (RR is 0-2.2 mmol/L) and an LDL cholesterol level of 2.28 mmol/L (RR is 3.9-5.2 mmol/L). Serum creatinine level gradually rose to 290 mmol/L; the C-peptide level was 137 pmol/L (RR is 256-1325 pmol/L). Abdominal ultrasound showed multiple cortical renal cysts with multiple stones, mild hepatomegaly, and multiple gall bladder stones. Molecular genetic testing was not available. His HbA$_{1c}$ level of 6.2% was controlled with insulin. He was diagnosed clinically as having Bardet-Biedl syndrome based on the clinical presentation of DM with retinitis pigmentosa, obesity, polydactyly, renal cortical cysts, and an ataxic gait.

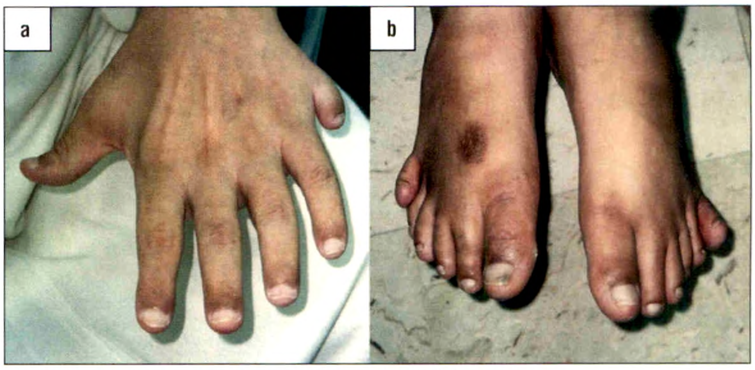

Figure 1. Polydactyly of left hand (a) and both feet (b).

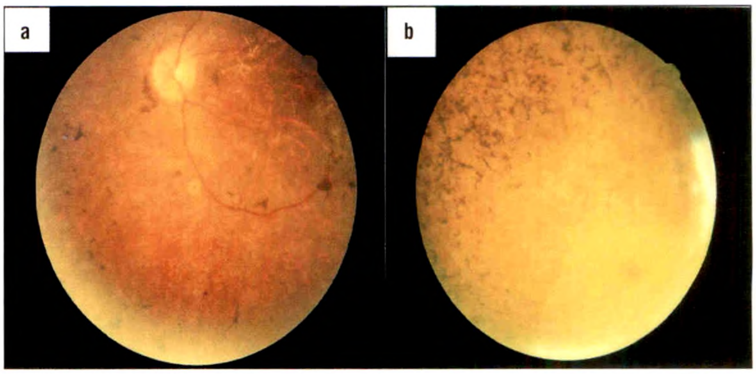

Figure 2. Retinitis pigmentosa: a speckling of the retinal pigment epithelium with bone-spicule pigmentation, optic nerve pallor, and retinal arteriolar attenuation.

CASE 2

34-year-old Kuwaiti male was referred to our diabetes clinic for insulin-treated diabetes and uncontrolled hyperglycemia. A detailed history revealed that diabetes was diagnosed five years ago along with a diagnosis of hypertension. He begin having gradual visual deterioration and night blindness at the age of six years and was registered as being blind at the age of nine years. His parents were third cousins and his sister, who was also diabetic and blind, had died of renal failure. He had been married for one year; primary infertility was also observed. Clinical examination revealed a weight of 71 kg, a height of 158 cm, a BMI of 28.4 kg/m^2, and a blood pressure of 120/80 mm Hg. Fundus examination revealed retinitis pigmentosa along with optic atrophy (Figure 2, Panel b). Systemic examination did not reveal any remarkable findings, instead showing intact peripheral sensation and pulsation. Investigations revealed a fasting blood glucose level of 18.1 mmol/L, HbA$_{1c}$ of 15.1%, and a C-peptide level of 2247 pmol/L (normal range: 256-1325 pmol/L). The triglyceride level was 4.06 mmol/L, LDL cholesterol level was 4.25 mmol/L, and HDL cholesterol level was 1.07 mmol/L (RR is 1.01-2.94 mmol/L). His serum creatinine level was 96 µmol/L and microalbuminurea testing yielded 457 mg/day (RR is <20 mg/day). The alkaline phosphatase level was 155 U/L ((RR is 50-136 U/L)) and ALT 63 U/L (RR is 30-65 U/L). Thyroid function tests were normal. The audiogram demonstrated sensineural deafness. Endocrinological evaluation revealed azospermia and biopsy showed only Sertoli cells. Genetic testing yielded 46XY, but no chromosomal mutations, deletions or other abnormalities could be detected. Molecular genetic testing was not available. His diabetes was gradually controlled with increasing doses of insulin and metformin given at doses of up to 2 g per day along with statins. This resulted in better glycemic control and significant improvement in the lipid profile and HbA$_{1c}$ level of 9.7%. Subsequently, metformin was discontinued because of elevated liver enzyme levels. Abdominal ultrasound revealed a fatty liver and liver biopsy revealed nonalcoholic steatohepatitis. Screening for hepatitis viruses B and C as well as for ANCA and ANA antibodies was negative. Following improvement of the liver profile, rosiglitazone was prescribed along with insulin, and HbA$_{1c}$ improved to 8.8%. Later, the renal profile was found to be impaired and ultrasound examination of the liver showed cirrhotic changes. The serum creatinine level remained stable at 120 µmol/L, and he declined renal biopsy. Rosiglitazone was discontinued and the insulin dose increased up to 245 units per day. His blood sugar levels were still out of control and he was not adherent to his controlled diet regimen. He was diagnosed clinically as having Alström syndrome based on his clinical presentation of insulin-resistant diabetes with retinitis pigmentosa, deafness, hepatic dysfunction, renal impairment, and hypogonadism.

DISCUSSION

RP is a group of inherited disorders characterized by progressive peripheral vision loss and night vision difficulties that can lead to central vision loss. It is caused by molecular defects in more than 100 different genes.[1] RP is most commonly found in isolation, but it can be associated with several systemic diseases such as deafness, neurological disorders, cardiovascular anomalies, renal manifestations, obesity, and diabetes. Four rare syndromes have been reported as describing the combination of RP and DM. These include Bardet-Biedl syndrome, Alström syndrome, Kearns-Sayre syndrome, and rarely, Wolfram syndrome. Table 1 summarizes the mode of inheritance, the affected genes, and the clinical manifestations of each syndrome.

The term, "Laurence-Moon-Bardet-Biedl" was coined in 1925.[2] In 1982, Laurence-Moon and Bardet-Biedl syndromes began to be considered as two separate syndromes due to the essential differences between them.[3] The Laurence-Moon syndrome is considered to be composed of retinitis pigmentosa, obesity, hypogonadism and spastic paraparesis without polydactyly, and DM. However, recently, and because mutations

Table 1. Syndromes associated with diabetes mellitus and retinitis pigmentosa

	Bardet-Biedl	Alström	Kearns-Sayre	Wolfram (DIDMOAD)
Mode of inheritance	Autosomal recessive	Autosomal recessive	Mitochondrial disease	Autosomal recessive
Genes involved	12 genes, mostly BBS1, BBS2, BBS6, BBS10, MKKS	ALMS1 gene, 81 mutations so far	mt-DNA mutations/deletions	WFS1 gene mutation
Clinical features				
Diabetes mellitus	Noninsulin-dependent	Insulin-resistant	Insulin-dependent	Type 1 nonautoimmune
Retinitis pigmentosa	+	+	+	+
Childhood obesity	+	+	-	-
Mental retardation	+	-	-	-
Polydactyly	+	-	-	-
Infertility/hypogonadism	+	+	+	-
Sensorineural deafness	-	+	-	+
Renal disease	+	+	-	-
Hepatic involvement	+	+	-	-
Short stature	±	+	+	-
Acanthosis nigricans	-	+	-	-
Cardiac disease	Congenital detects	Cardiomyopathy	Heart block	-
Neurologic disease	Ataxia, incoordination	Absence seizures, sleep disorders	External ophthalmoplegia	Ataxia, brain stem disorder, nystagmus, sleep apnea
Other features	-	Hypothyroidism, hypertension	Hypothyroidism Hypomagnesemia, Hyperaldosteronelism	-
Prognosis	Death is usually due to renal failure and 25% die by the age of 44 years	Death is usually due to renal failure and patients rarely live longer than 40 years.	Death is usually from cardiac disorders and death is common in the third or fourth decade of life	Death is usually due to respiratory and renal failure and median age at death is 30 years

Table 2. Diagnostic criteria for Alström syndrome and Bardet-Biedl syndrome.

	Major criteria/Primary features	Minor criteria/Secondary features	Other supportive evidence	Diagnosis
Bardet-Biedl syndrome	Cone-rod dystrophy Postaxial polydactyly Truncal obesity Learning disabilities Hypogonadism in males or genital abnormalities in females Renal anomalies	Speech disorder/delay Strabismus/cataracts/astigmatism Brachydactyly/syndactyly Developmental delay Polyuria/polydipsia (nephrogenic diabetes insipidus) Ataxia/poor coordination/ imbalance Mild hypertonia (especially lower limbs) Diabetes mellitus Dental crowding/hypodontia/small dental roots/high-arched palate Cardiovascular anomalies Hepatic involvement		4 major criteria OR 3 major + 2 minor criteria
Alström syndrome	ALMS 1 mutation in 1 allele and/or family history of Alström syndrome Vision (legal blindness, history of nystagmus in infancy/childhood, cone and rod dystrophy by electroretinogram	Obesity and/or insulin resistance and/or type 2 diabetes History of dilated cardiomyopathy/ congestive heart failure Hearing loss Hepatic dysfunction Renal failure Short stature Males – hypogonadism Females – irregular menses and/or hyperandrogenism	Recurrent pulmonary infections Normal digits History of developmental delay Hyperlipidemia Scoliosis Flat wide feet Hypothyroidism Hypertension Growth hormone deficiency Alopecia Recurrent urinary tract infections or urinary dysfunction	2 major + 2 minor criteria OR 1 major + 4 minor criteria

have now been detected in Bardet-Biedl syndrome genes in families conforming to the diagnosis of the Laurence-Moon syndrome, some authorities do not believe in this distinction and consider the two to be one syndrome under the label of Bardet-Biedl syndrome (BBS). The prevalence of BBS ranges from one in 13 500 in a consanguineous population, to one in 160 000 in nonconsanguineous populations. It is usually inherited in an autosomal recessive manner.[2] It is clinically characterized by: (i) cone-rod dystrophy (100%) that usually affects the vision in the first decade; the patients will be legally blind by the second decade, (ii) obesity (72-96%), which usually begins within the first year, (iii) postaxial polydactyly (58-69%), (iv) renal dysfunction (46%), (v) cognitive impairment, (vi) male hypogonadotrophyic hypogonadism, and (vii) complex female genitourinary malformations. All above manifestations are postulated to be due to defects in the cilia. Because of the wide range of clinical variability observed within BBS, the diagnosis is established on the basis of clinical findings. Clinical diagnosis can be made with four primary or three primary and two secondary features as shown Table 2.[2] Diabetes mellitus is diagnosed in 32-45% of cases with BBS.[3,4] It is usually noninsulin-dependent diabetes but is occasionally insulin-dependent, and is treated as usual in the general population.[2,4] Renal disease is a major cause of morbidity and mortality in BBS patients and 25% die by the age of 44 years.[4] Molecular genetic studies found 14 genes to be associated with BBS. [2,5,6,7] Table 3 summarizes the genes identified so far and the percentage of the most common causative mutations and exons. However, approximately 20% to 30% of patients do not have identifiable mutations, suggesting that more BBS cases are yet to be identified. Some genotype/phenotype correlations have been reported (Table 3) although most of the studies did not confirm these correlations.

Alström syndrome is a rare autosomal recessive genetic disease characterized by multi-organ dysfunction. It is clinically characterized by: (i) childhood obesity (95%), (ii) progressive cone-rod dystrophy ultimately leading to blindness with 90% of the patients becoming blind at the age of 16 years, (iii) sensorineural deafness (80%), (iv) dilated cardiomyopathy (60%), (v) heptic

Table 3. Known genes and gene mutations in Bardet-Biedl syndrome and Alström syndrome.

	Causative genes identified	Mutations (%)	Exon (%)	Phenotype correlations
Bardet-Biedl syndrome	14 genes[5]			
	BBS1[2]	- 11q13 (23.2%)[2]	- 12 (46.7%), 16 (16.7%), 15 (6.7%), 11 (6.7%), 4 (10%), 13 (3.3%), 10(3.3%), 8(3.3%),1(3.3%)[2]	
	BBS2[2]	- 16q21 (8.1%)[2]	- 2 (30%), 1 (15%), 4 (15%), 8 (10%), 6 (10%), 14 (10%), 9 (5%), 15 (5%), 3 (5%)[2]	- BBS2, BBS3, and BBS4 correlated with characteristic ocular phenotypes. BBS4 correlated with extra digits. BBS2, BBS3, BBS4, and BBS6 correlated with adult obesity. BBS4 and BBS6 correlated with childhood obesity.
	ARL6/BBS3[2]	- 3p12-q13 (0.4%)[2]		
	BBS4[2]	- 15q22.3-q23[2]	- 13 (16.7%), 12 (16.7%), 8 (16.7%), 4 (16.7%), 3-4 (16.7%), 15 (8.3%), 7 (8.3%)[2]	
	BBS5[2]	- 2q31 (0.4%)[2]	- 7	
	MKKS/BBS6[2]	- 20p12 (5.8%)[2]	- 3 (80.7%0, 6 (12.9%), 4 (3.2%), 5 (3.2%)[2]	
	BBS7[2]	- 4q27 (1.5%)[2]	- 10 (50%), 6 (25%), 7 (25%)[2]	
	TTC8/BBS8[2]	- 14q32.1 (1.2%)[2]	- 10[2]	
	B1/BBS9[2]	- 7p14 (NK)[2]		
	BBS10[2]	- 12q21.2 (20%)[2]		
	TRIM32/BBS11[2]	- 9q31-q34.1 (<0.4%)[2]		
	BBS12[2]	- 4q27 (5%)[2]		
	MKS/BBS13[2]	- 17q23[6]		
	MKS/BBS14[2]	- 12q21.3[7]		
Alström syndrome	81 mutations[8] ALS1	2p1[2]	- 16 (40%)[8] - 10 (23%)[8] - 8 (21%)[8] - 6[8] - 12[8] - 17[8] - 18[8]	Exon 16 correlated with more severe disease, early RP, urologic dysfunction, dilated cardiomyopathy, and diabetes. Axon 8 is correlated with renal disease.

ARL6, ADP-ribosylation factor-like 6 gene; MKKS, McKusick-Kaufman syndrome; TTC8, Tetratricopeptide repeat domain 8 gene; TRIM32, Tripartite motif-containing 32 gene; MKS, Meckel-Gruber syndrome[2,5,6,7,8]

dysfunction (80%), (vi) renal insufficiency (50%), and (vii) endocrinological features.[8] It was first described in 1959 and 500 cases have been detected globally so far without any gender predilection.[8] It is now considered to be one of the class of genetic diseases known as ciliopathies in which the ciliary function of various cell types are affected, resulting in dysfunction of cells of renal tubules, sperm cells, and retinal cells, thereby producing the components of the syndrome.[9] The genetic disease is due to mutations in the *ALMS1* genes, of which 81 mutant genes have been detected so far (Table 3).[8] As in Bardet-Biedl syndrome, and because of the wide-ranging and complex spectrum of phenotypes reported in the literature, Marshall et al adopted the diagnostic criteria for Alström syndrome as shown in Table 2.[10] In adults, two major criteria and two minor criteria, or one major and four minor criteria are required for the clinical diagnosis of the syndrome. Hyperinsulinemia, which develops in early childhood, has been reported in 92% of individuals with ALM. Type 2 DM is diagnosed in 82% of the affected individuals older than 16 years of age and it is usually diagnosed in the second or third decade.[8,11] Hyperinsulinemia and hyperglycemia may be managed with a low carbohydrate diet, metformin, and rosiglitazone.[12,13] Cognitive function is preserved in Alström syndrome and polydactyly is not a feature, thus distinguishing it from Bardet-Biedl syndrome. Dilated cardiomyopathy can occur at any age, but it mainly occurs in infancy and is the main cause of mortality in that age group, whereas renal failure is the main cause of mortality in elder patients.[14] The life span of patients with Alström Syndrome rarely exceeds 40 years.[13]

Kearns-Sayre syndrome (KSS) is a rare mitochondrial disease with an as yet undetermined mode of inheritance. The true prevalence of this syndrome is unknown. The mitochondrial abnormalities involve mitochondrial DNA mutations, rearrangements, or deletions. It was first described in 1958 and several hundred cases have been diagnosed to date. Its labile clinical features make it rather difficult to diagnose.[15] It is characterized by: (i) external ophthalmoplegia, (ii) retinitis pigmentosa, (iii) cardiac conduction defects, (iv) a variety of endocrine abnormalities including short stature, gonadal failure, diabetes mellitus, thyroid disease, hyperaldostronism, hypomagnesemia, and (v) bone and dental abnormalities.[16] Diabetes mellitus was recorded in 13% of cases with KSS,[16] and was of the insulin-dependent type due to insulin deficiency.[17] KSS cases have high mortality rates which is directly attributable to the cardiovascular manifestations of this syndrome.[18]

Wolfram syndrome (WFS) is an autosomal recessive neurodegenerative disorder that predisposes individuals to the development of type 1 diabetes in association with progressive optic atrophy. It is an extremely rare condition with a prevalence of 1 in 770 000.[19] It is also known as DIDMOAD syndrome, the name being derived from diabetes insipidus, diabetes mellitus, optic atrophy, and deafness. RP was reported in a few cases.[20] The genetic basis of the disease is due to mutations in the WFS1 gene, which has been postulated to be an endoplasmic reticulum calcium channel transporter in pancreatic β-cells and neurons.[18] Although diabetes and optic atrophy represent the minimal diagnostic criteria, other clinical characteristics include neurodegenerative disease involving the hypothalamus, brain stem, and the cerebellum. Diabetes is usually diagnosed in childhood and is of the nonautoimmune insulin-dependent type.[21] Deafness is not always a prominent component. Other abnormalities include dilated renal outflow tracts and primary gonadal atrophy. Death is usually due to respiratory failure from brain stem involvement[22] and renal failure.[19]

Of note, patients with DM and RP do not develop signs of diabetic retinopathy. The absence of diabetic retinopathy in RP was explained by the suggestion that the loss of photoreceptors reduced glycolysis and decreased the production of free radicals.[23] The combination of diabetes and blindness in these syndromes confers a high risk of foot ulceration. However, it has been reported that patients with Alström syndrome are protected from peripheral sensory neuropathy.[24]

In conclusion, we report two cases who had presented with retinitis pigmentosa and diabetes mellitus along with other overlapping clinical manifestations. Both cases were diagnosed based on clinical features into two different pleiotropically genetic disorders, namely, the Bardet-Biedl syndrome and Alström syndrome. Due to the overlapping clinical manifestations in these genetic disorders and the cost and time usually needed for genetic studies, clinical criteria can be used for diagnosis and as a guide for genetic mapping in affected individuals. Genetic studies are needed to confirm diagnosis, detect carriers in at-risk family members, and for genetic counseling and prenatal diagnosis.

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case report

Hydrogen sulfide exposure in an adult male

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Hydrogen sulfide (H_2S) is responsible for many incidents of occupational toxic exposure, especially in the petroleum industry. The clinical effects of H_2S depend on its concentration and the duration of exposure. H_2S is immediately fatal when concentrations are over 500-1000 parts per million (ppm) but exposure to lower concentrations, such as 10-500 ppm, can cause various respiratory symptoms that range from rhinitis to acute respiratory failure. H_2S may also affect multiple organs, causing temporary or permanent derangements in the nervous, cardiovascular, renal, hepatic, and hematological systems. We present a case of occupational exposure to H_2S leading to multi-organ involvement, acute respiratory failure, organizing pneumonia, and shock resembling acute sepsis. The patient also developed mild obstructive and restrictive pulmonary disease and peripheral neuropathy.

Hydrogen sulfide (H_2S) is responsible for many incidents of occupational toxic exposure, especially in the petroleum industry. The clinical effects of H_2S depend on its concentration and the duration of exposure. H_2S is immediately fatal when concentrations are over 500-1000 parts per million (ppm).

Hence, H_2S has been referred to as the "knock down gas" because inhalation of high concentrations can cause immediate loss of consciousness and death.[2,3] However, prolonged exposure to lower concentrations, such as 10-500 ppm, can cause various respiratory symptoms that range from rhinitis to acute respiratory failure.

There are many cases of H_2S exposure in the agricultural industry [4] and their prevalence has increased markedly with the development of porcine confinement facilities.[1,5] H_2S is the primary chemical hazard of natural gas production.[6] We report a severe case of hydrogen sulfide (H_2S) intoxication. The patient survived long enough to observe the sequelae of this entity, which can include neuropsychiatric morbidity.

CASE

The patient was a 31-year-old male who worked in an oil refinery. He was brought to the emergency department with fever (a temperature of 39.3°C) and respiratory symptoms. He was hypotensive with a blood pressure of 68/40 mm Hg. He reported that he had been welding in a large container used for the storage of sulfur compounds in an open space before the onset of symptoms. No other chemical compounds were used and the container was clean at that time, but there were some unknown fluid residues on the floor. At the beginning of the welding process, white fumes with a "rotten egg" odor emanating from the container. The patient immediately felt dizzy and developed rhinorrhea, teary eyes, nausea, and shortness of breath, chest tightness and cough. These symptoms increased over the following hours followed by hemoptysis. He was seen by first aid providers and was removed from the scene, given oxygen, and was transported to the emergency room. There were no other workers in the same place during the event of the poisoning. He was using his personal protective equipment, including gowns and a mask.

Subsequently, the patient was admitted to the intensive care unit. On examination, he was found to be hypotensive (BP 68/40 mm Hg) and tachypneic (respiratory rate of 26/min). The neck was supple without any lymphadenopathy. A chest examination revealed bilateral rhonchi, but examination of the heart and abdomen found no abnormalities. Neurologically, he was combative and confused initially and became lethargic and obtunded later. There were no skin lesions. Shortly after admission, the patient developed acute respiratory failure requiring mechanical ventilation. Chest radiography (CXR) showed right pleural effusion and consolidation (Figure 1). Initial and subsequent laboratory (Table 1) revealed signs of ischemic cardiac injury, abnormal coagulation profile, renal insufficiency, and slight leukocytosis. Arterial blood gas showed a pH of 7.34; PCO_2: 44 mm Hg; PO_2: 77 mm Hg; and oxygen saturation: 95% on an inspired oxygen of 35%. Other laboratory data were as follows: BUN 43 mEq/L, creatinine 2.6 mg/dL, Na 135 mEq/L, K 4.8 mEq/L, Cl^- 105 mEq/L, and CO_2 19 mEq/L.

As the patient was hypotensive, he was resuscitated with intravenous fluid and vasopressors. Intravenous hydrocortisone was started for chemical pneumonitis, but it was stopped after four days because there were no signs of improvement. Infections were ruled out and empirical broad-spectrum antibiotics were subsequently discontinued. All serological studies were negative, including *Mycoplasma*, *Legionella*, and HIV. A thoracentesis revealed an exudative pleural fluid. The gram stain showed many cells, 90% neutrophils, no organisms, and the cultures were negative. Cytology on bronchoalveolar lavage showed a few cells consistent with herpes simplex infection, thought to be a contamination from an upper airway and nasal infection. After several days of supportive care, the patient became hemodynamically stable with improved cardiac function and was extubated successfully.

However, he continued to have a right lower lobe consolidation (Figures 2a and 2b) despite appropriate antimicrobial therapy, including acyclovir. Lung biopsy via video-assisted thoracoscopic surgery was performed and showed diffuse alveolar damage with organizing pneumonia. Special stains for herpes viruses were negative. Sections of the lung showed the presence of numerous alveolar spaces lined by reactive pneumocytes type II. Many of the alveolar spaces were filled with an admixture of macrophages, scattered eosinophils, and neutrophils. In addition, several alveoli showed clumps of proliferating fibroblasts admixed with histiocytes and other inflammatory cells, denoting the presence of organizing pneumonia. He was restarted on intravenous hydrocortisone and showed a remarkable response with significant improvement in respiratory symptoms and radiographic findings (Figure 3).

About 40 days after the incident, spirometery revealed a mild obstruction with an insignificant response to bronchodilators. Lung volume showed mild restriction and the diffusion capacity was at low-normal levels after correction for alveolar volume. The findings were consistent with mixed restrictive and obstructive pulmonary disease. He developed grayish nail-bed discoloration, suggestive of an exposure to a sulfur compound. Neurological evaluation, including an electromyography, revealed evidence of peripheral neuropathy. A follow-up chest x-ray after discharge showed complete resolution of his pulmonary infiltrate.

Table 1. The patient's laboratory data over the hospital course.

	Day 1	Day 2	Day 3 (ICU admission)	Day 4	Day 7
WBC (k/mm^3)	14.7	1.5	1.6	8.5	22.9
Neutrophils %	88	21	8	16	70
Hemoglobin (g/dL)	13	12.7	13	12.4	11.5
Platelets (k/mm^3)	289	237	223	202	294
BUN (mg/dL)	-	-	43	25	31
Creatinine (mg/dL)	-	-	2.6	1.1	0.9
INR (ratio)	-	-	1.9	1.3	1
PTT (seconds)	-	-	44	49	32
Troponin I (ng/mL)	-	-	37.6	9.4	<0.5
EKG	-	-	Inferior infarct	-	Normalized
Echocardiogram	-	-	EF 30%, impaired LV wall motion	-	EF>55%, LV function normalized

BUN=Blood urea nitrogen; INR=International ratio; PTT=Partial thrombin time; EKG=Electrocardiogram

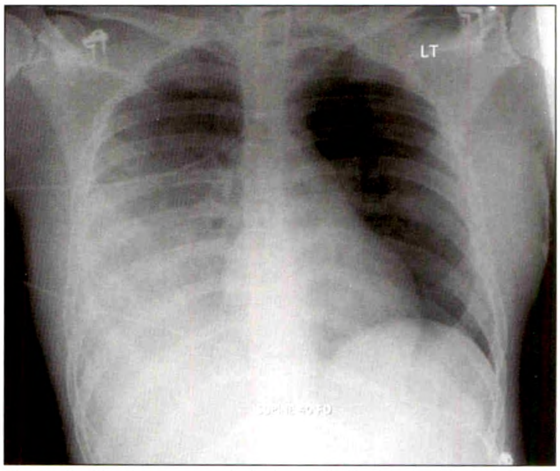

Figure 1. Chest x-ray on admission to ICU, showing right consolidation and pleural effusion.

DISCUSSION

Hydrogen sulfide (H_2S) is the primary chemical hazard of natural gas production.[6,7] In a retrospective review

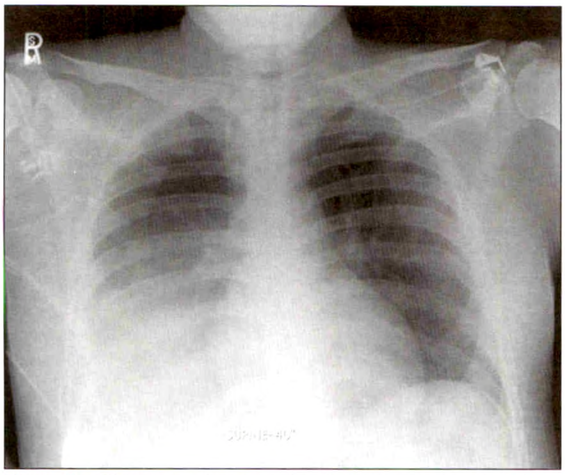

Figure 2a. Chest x-ray three weeks after presentation, showing persistent right lower lobe consolidation.

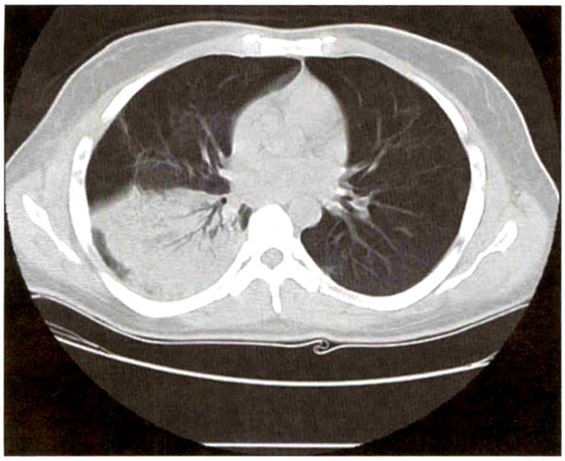

Figure 2b. Chest CT scan, three weeks after presentation, showing persistent right lower lobe consolidation.

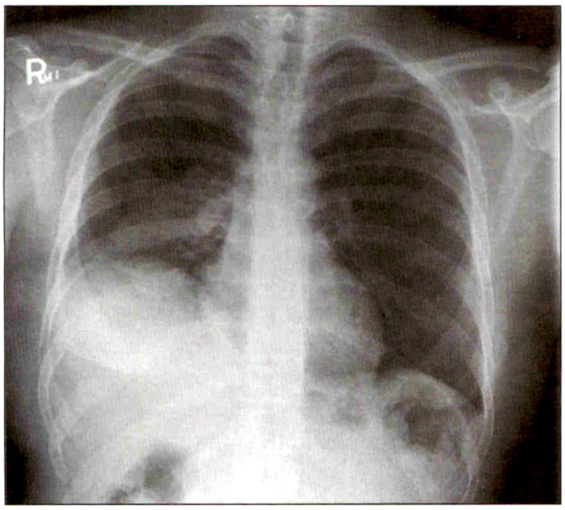

Figure 3. CXR, two days after initiating high dose steroids following lung biopsy, showing significant improvement in right lower lobe consolidation.

from oil and gas industry in Canada revealed 221 cases of H_2S exposure from 1969 to 1973, and 173 patients were transported to the hospital; 14 victims (6%) were dead on arrival.[8]

The findings in this case are consistent with exposure to H_2S. There was a history of an odor of rotten eggs emanating from residues in the work site that was suggestive of H_2S with related symptoms and clinical findings, as described previously.[9-11] H_2S is a colorless gas with a characteristic odor.[12] However, persistent exposure to air concentrations above 100 ppm produces olfactory fatigue, which impairs the ability to detect the characteristic odor of rotten eggs.[12]

Following the inhalation accident, the patient developed multi-organ involvement simulating sepsis: acute respiratory failure, obtundation, leucopenia, neutrophilia, abnormal coagulation profile, renal insufficiency, shock, cardiac injury, and reduced cardiac output (an ejection fraction of 30%).[9,13] A persistent pulmonary infiltrate proved to be an organizing pneumonia. Organizing pneumonia is characterized by the presence of granulation tissue in the distal air spaces consisting of fibroblasts–myofibroblasts embedded in connective tissue.[14] When organizing pneumonia is an associated feature, the term, "bronchiolitis obliterans" is added. Bronchiolitis obliterans organizing pneumonia (BOOP) may follow pulmonary infection, drug toxicity, or may appear in the context of connective tissue diseases or after lung or bone marrow transplantation.[15] Cryptogenic organizing pneumonia (COP), the idiopathic form of organizing pneumonia (also known as idiopathic BOOP), is a distinct clinical entity. COP has the predominant features of pneumonia, rather than a primary airway disorder.[14] The mainstay of treatment of BOOP is with a corticosteroid resulting in a rapid clinical improvement and clearing of the opacities on chest imaging without significant sequelae.[14,16]

Injury due to H_2S exposure occurs primarily by inhalation. Once absorbed, the compound is distributed in the blood and taken up by the brain, liver, kidney, pancreas, and small intestines. Sulfur compounds are severely irritating to the respiratory tract, leading to rhinorrhea, sneezing, sore throat, wheezing, shortness of breath, chest tightness, hemoptysis, and a feeling of suffocation.[1] Sulfur compounds can cause leucopenia and neutropenia,[9,13] as well as cardiac injury with elevation of troponin I and creatine kinase.[9,10] The mechanism of H_2S toxicity is related to inhibition of oxidative phosphorylation, which causes a decrease in the available cellular energy. A phenomenon referred to as "knockdown" was reported in oil field workers to describe a sudden, brief loss of consciousness associated with amnesia, followed by immediate full recovery. This phenomenon usually occurs after short-term exposure to very high concentrations of H_2S.[17]

Various pulmonary complications may follow inhalation injury. In a study of 203 patients with first- to third-degree burns, lung complications developed in 7.8%, leading to adult respiratory distress syndrome (ARDS) in 5.4%.[18] Organizing pneumonia may occur following inhalation of toxic fumes and chemicals. These pathological changes are relatively well-known for patients who have been exposed to relatively high concentrations of any slightly water-soluble, toxic, inhaled compound (not unique to H_2S) and may represent a spectrum of inhalation concentrations and severity. It is interesting to note that unilateral pulmonary abnormalities occurred in the present case. Similarly, in the study cited above, 36% of the patients had only right lung involvement.[18] The presence of leucopenia and neutropenia in the present case is also interesting. In a study of chronic exposure to H_2S, the absolute mean numbers of white blood cells, lymphocytes, and neutrophils were seen to be significantly decreased in the exposed group compared with the control.[19]

The patient showed evidence of neuropathy on follow-up visits. Annual neurological and neuropsychological testing for at least five years is recommended for patients with H_2S exposure because of the potential chronic neurological sequelae.[20] Other reports suggest that temporary memory loss, attention deficits, blunted affect, permanent retrograde amnesia, executive function deficits, slowing in central information processing, and planning deficits may occur in such patients.[17]

The mainstay of therapy is supportive care. There are reports that suggest early administration of hyperbaric oxygen, amyl nitrite, and sodium nitrite may be beneficial.[2,21] Amyl nitrite-induced methemoglobinemia is due to competitive binding of the hydrosulfide anion. This effect presumably reactivates and protects cytochrome oxidase.[22] However, one of the toxic effects of H_2S is the inhibition of cytochrome oxidase. Nitrites produce methemoglobin which has a higher affinity for H_2S than for cytochrome oxidase. The resulting sulfmethemoglobin eventually returns to hemoglobin. Hyperbaric oxygen in a few case reports and animal studies may work as an adjuvant treatment in patients with persistent neurological injury or oxygenation defects.[23] Therapeutic red cell exchange may also be used to treat H_2S toxicity similar to the treatment of aniline, arsine, chloramines, carbon monoxide, cyanide, and, methemoglobinemia.[24]

As H_2S is a potential problem in the transport and storage of crude oil, preventive measures are extremely important in preventing lethal exposure to hydrogen sulfide toxicity. Personal protective equipment should include safety glasses, respiratory protection or equipment, and long-sleeved shirts.[25] In addition, limiting exposure at the work place and the use of a personal safety gas detector may aid in the protection of employees working with potential gas exposure. In a retrospective analysis, 77 of the 80 deaths were thought to be potentially preventable with the use of an H_2S alarm or portable meters.[26]

In conclusion, we report a case of an occupational exposure to H_2S leading to acute respiratory failure, multi-organ involvement simulating sepsis, and organizing pneumonia. The diagnosis of hydrogen sulfide poisoning relies mainly on the clinical presentation and exposure. H_2S poisoning may lead to persistent neuropsychiatric morbidity. The treatment remains generally supportive and amyl nitrites may be beneficial.

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Solitary coronary artery nourishing the entire heart

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Coronary artery anomalies are being more frequently diagnosed these days both because increasing numbers of patients are undergoing diagnostic studies and because advanced radiographic imaging methods are now commonly available. An isolated single coronary artery giving rise to the main coronary branches is a rare congenital anomaly. In this report we present a patient with a solitary coronary ostium, with both the left and right coronary artery systems arising from it, and then following their usual courses. This case was diagnosed incidentally during conventional angiography.

Coronary anatomy is an important issue during the management of a wide range of congenital and acquired cardiovascular pathologies. Knowledge of normal coronary physiology and of the variations in anatomy are vital prior to surgical interventions on the heart. Good operative outcome mostly depends on a thorough understanding of the coronary circulation.

Today, variations in coronary anatomy are frequently detected as a result of the availability of better screening systems and because an increasing number of patients are seeking solutions for cardiovascular disorders. Although variant conditions are infrequent[1-10] there is much heterogenicity in the coronary vasculature, and as a result there is no well-established classification system in the literature and no clear-cut guidelines on whether to treat or not to treat these variant conditions. In this report, we present a 54-year-old patient with a solitary coronary ostium giving rise to the right and left main coronary arteries, with the latter supplying blood to the entire heart. Informed consent was obtained from the patient for publication of this case report.

CASE

The patient was a 54-year-old male who presented to the clinic with chest pain and dyspnea on exertion. He had history cigarette smoking (1 pack/day for 30 years). His past history was otherwise unremarkable. Family history included death of the father at the age of 45 years, most probably due to a myocardial infarction. However, his elder male siblings were alive and free of any symptoms. His electrocardiography (ECG) was normal. He underwent a stress ECG, which had to be stopped at the third minute of maximal effort capacity because the patient reported fatigue and dyspnea. Angiography was decided upon for further diagnostic workup.

Coronary angiography was performed using the traditional Judkings method.[11] The left coronary system was completely normal. Moreover, it gave rise to the right coronary artery (Figures 1 and 2). Another check to visualize the right coronary artery in the right coronary sinus did not reveal the right coronary ostium as expected. Ventriculography indicated minor inferior wall dyskinesia. Echocardiography confirmed the inferior wall motion abnormality and revealed mild aortic and mitral insufficiencies.

We recommended aspirin 300 mg daily and cessation of smoking and advised him to come for yearly follow-ups. Further nuclear or radiographic studies such as coronary CT angiography or MR imaging were not done.

DISCUSSION

Coronary artery anomalies are rare. They are seen in

0.3% to 1.3% of patients undergoing coronary angiography[1] and in 0.17% of routine autopsy studies.[2] In the younger age groups, they may be responsible for 4% to 15% of cases of sudden death.[2,3]

An isolated single coronary artery is a solitary coronary vessel arising from the ascending aorta, giving rise to the major coronary branches and thus nourishing the entire heart. In effect, both the right coronary artery and the left main coronary artery arise from a single aortic sinus.[6] The anomaly is usually diagnosed incidentally during coronary artery angiograms or on postmortem evaluations. The incidence of single coronary artery is approximately 0.019% to 0.4% in the population.[4-6] They reportedly constitute <3% of all coronary anomalies.[1] They are frequently found in association with other congenital cardiac lesions. In adult subjects, the clinical interest relates to their occasional association with myocardial ischemia, congestive heart failure, and/or sudden death. Additionally, the presence of coronary artery anomalies may pose special problems during coronary angiography, interventional procedures, and coronary artery bypass surgery.

There is no gender preference for coronary artery anomalies.[3,4] The well-known ALCAPA (anomalous origin of left main coronary artery from the pulmonary trunk) syndrome, when significant, may result in symptoms during young adulthood; otherwise the syndrome is generally clinically silent and is discovered only incidentally during diagnostic studies for other pathologies. The exact incidence of clinical events due to coronary artery anomalies is not known as most cases are clinically silent and the subject's quality of life and life expectancy are unaffected.

A single coronary artery may be diagnosed by various imaging modalities, including CT, MR imaging, echocardiography, and conventional coronary angiography.[6,7] The advances in radiologic diagnostic methods, especially in CT and MR imaging, has allowed the origin and course of anomalous coronary arteries to be demonstrated with excellent resolution. However, coronary angiography is still the gold standard diagnostic tool, enabling simultaneous percutaneous interventions for treatment of associated or non-associated coronary lesions.[7]

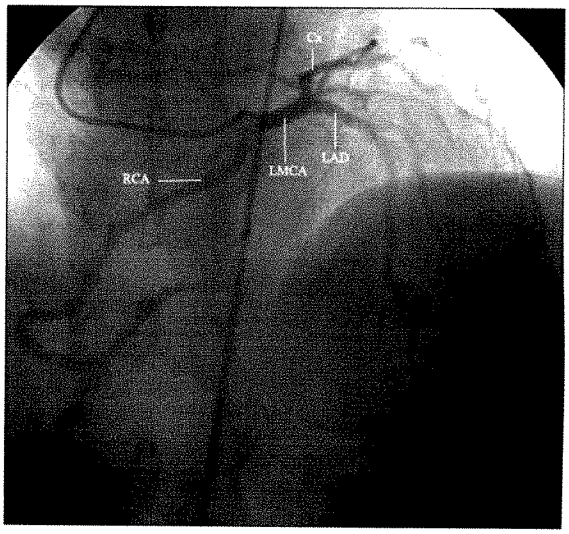

Figure 1. Coronary angiography, right anterior oblique view with a caudal projection. Note the single coronary ostium and the right coronary artery and left main coronary artery originating from the solitary vessel. LMCA: Left main coronary artery; LAD: left anterior descending coronary artery; RCA: right coronary artery; Cx: circumflex coronary artery.

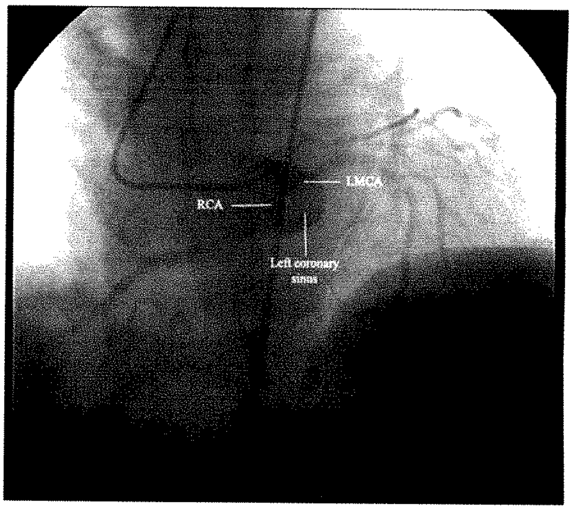

Figure 2. Angiographic imaging of left coronary sinus showing a single coronary ostium. LMCA: Left main coronary artery; RCA: right coronary artery.

A single coronary artery may appear in various forms. Smith, in the mid-20th century, classified single coronary artery anomalies into three major groups.[9] In the first type, the single coronary artery follows the course of the right coronary artery, continues into the circumflex artery and then as the left anterior descending coronary artery; alternatively, there may be a single left main artery that branches into the left anterior descending artery and circumflex artery, the latter extending across the crux to form the right coronary artery. In the second type, after its origin the main trunk divides into the right and left main arteries (as in our patient) or into right coronary artery, left anterior descending coronary artery, and circumflex artery. In the last type, the single coronary artery branches atypically and so there is little similarity between the coursing of the three major arteries.

There is no consensus regarding the risk for atherosclerosis in casees of single coronary artery. The literature includes variable reports. Porto et al. claim that there is an increased risk of atherosclerosis in the case of a single coronary artery, which may result from acute-angle takeoff malformation.[10] Coronary spasm,[12] angina pectoris, and myocardial infarction are also reported in the absence of coronary stenosis.[13] The literature includes reports of percutaneous angioplasty and stenting in single coronary artery.[10] In contrast, according to Garg et al.[14] single coronary artery anomaly is not associated with increased risk for the development of atherosclerotic coronary artery disease. In our case, the patient presented with chest pain and dyspnea on exertion. Additionally, there was minor inferior wall dyskinesia, which may be also associated with coronary anomaly.

In conclusion, a single coronary artery is a rare congenital anomaly. In the case presented, the right coronary sinus ended blindly. All the coronary arteries originated from a solitary coronary vessel and then followed their usual courses in the myocardium. We believe with the presentation.

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Gilbert syndrome in Iran, Fars Province

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To the Editor: Gilbert syndrome is a relatively common disorder of bilirubin metabolism that presents as unconjugated hyperbilirubinemia with otherwise normal liver structure and function.[1,2] It needs no treatment or long-term medical attention. It is usually one of the differential diagnoses of liver or hemolytic disease, and could be mistaken as such based on liver function tests. The primary cause of this syndrome is decreased hepatic levels of glucoronosyl transferase. It occurs in about 3% to 7% of the population,[1] but its prevalence differs by ethnicity. For example, in Saudis it was 3.6%,[3] in Singapore 3.2%,[4] and in Kashmir 3%.[5] However, in one report from Germany, the prevalence was 12.4% in men and 4.8% in women.[6] Reported ratios of male-to-female range from 1.5:1 to 7:1. Diagnosis of Gilbert syndrome is usually based on history, the findings of physical examination and some diagnostic tests such as phenobarbital, nicotinic acid, and rifampin tests, and specific genetic studies that are performed in special laboratories in some developed countries. Although genetic testing may be helpful for individual patients, the screening value of such genetic tests cannot be fully determined until accurate data are available for the prevalence and peneterance of the Gilbert syndrome genotype. Thus, genetic testing for Gilbert syndrome cannot be routinely recommended. To our knowledge, the prevalence of Gilbert syndrome has not yet been studied in the Iranian population. Hence, we designed this study to determine the prevalence rate in Iranian people of the Fars province.

We evaluated all persons who were referred for premarriage counseling or checkup to the Counseling Centre of Shiraz University from February 2007 to February 2009. Volunteers were included in our study after the details of our study were described to them. The history of blood and liver diseases were obtained from all of them and physical examination was done to rule out any stigmata of liver diseases. Complete blood and reticulocyte counts and liver function tests were performed for all the patients. Finally, a total of 460 persons whose history, physical examination, and laboratory tests revealed neither hemolytic nor liver disease, were taken as our subjects.

An overnight rifampin test was done for all patients wherein total serum and unconjugated bilirubin levels were measured from two blood samples. The first was taken before administering rifampin and the second, four hours after a single 600 mg oral dose of rifampin while the patient was fasting. Our criteria for the diagnosis of Gilbert syndrome were a total serum bilirubin level of more than 2.4 mg/dL and a serum unconjugated bilirubin level of more than 1.3mg/dL. These values have a high degree of sensitivity and specificity for the diagnosis of Gilbert syndrome according to the findings of previous studies.[1,7]

Our population comprised of 460 persons (227 male, 233 female), 88 persons of whom had Gilbert syndrome (58 male, 30 female) according to our criteria. The calculated prevalence of Gilbert syndrome was 19.1% in this population [Table 1]. The prevalence in male and females was 25.6% and 12.8%, respectively for a male-to-female ratio was 2:1. Based on our findings, we conclude that Gilbert syndrome is a common entity in Iran probably due to the higher number of consanguinous marriages in Iran, the method of screening (rifampin test), and the high genetic susceptibility in Iran.

Table 1. Number of people according to rifampin test results.

	Positive rifampin test	Negative rifampin test
Female	30	203
Male	58	169
Total	**88**	372

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Estimated average glucose: A new term in diabetes control

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To the Editor: Glycated or glycosylated hemoglobin (HbA$_{1c}$) levels have been used in planning and assessing the management of diabetic patients for the past couple of decades. Clinical trials have established the correlation between HbA$_{1c}$ and the development of diabetes complications and patient outcomes.[1,2] HbA$_{1c}$ results are expressed as the percentage of hemoglobin that is glycated and reflects the average blood glucose control over a period of approximately three months. In contrast, blood glucose levels are expressed in milligrams per deciliter and are used for daily monitoring by the patient and healthcare professionals. The discrepancy between HbA$_{1c}$ and blood glucose level units has been problematic and has created some confusion among patients. To reduce this confusion, researchers have determined and reported a linear correlation between HbA$_{1c}$ and self-monitored glucose levels obtained by frequent fingerstick capillary glucose testing and continuous glucose monitoring. A mathematical relationship between the average glucose level over the preceding three months and levels of HbA$_{1c}$ has been established,[3] resulting in the translation of HbA$_{1c}$ results into estimated average glucose (eAG). This approach was adopted by the American Diabetes Association (ADA).[4,5]

The estimated average glucose (eAG) converts the diabetic patient's HbA$_{1c}$ percentage point into an average blood glucose level in the units of measure seen by the patient on glucose meters for daily self-monitoring (mg/dL). Similar to HbA$_{1c}$, eAG evaluates a patient's overall success at controlling glucose levels and helps patients understand the monitoring of their long-term treatment. The relationship and association between HbA$_{1c}$ and eAG has been supported by several studies with the largest being published by the HbA$_{1c}$-Derived Average Glucose (ADBG) Study Group.[3] The ADAG linear regression formula that is used to calculate the eAG from A$_{1c}$ results is: "28.7×A$_{1c}$-46.7", and therefore, every one percent increase in HbA$_{1c}$ is approximately equivalent to an increase of 29 mg/dL in eAG (Table 1).[4,6]

As a clinical pharmacist practising in an ambulatory care setting, I have noted that patients find it difficult to explain and relate the HbA$_{1c}$ percentage of hemoglobin that is glycated to glucose measurements that they encounter through home or lab glucose monitoring ("numbers are not matching"). Since the introduction of eAG, I have used it to describe three-month blood glucose control in an easily comprehensible way that can be correlated to their home glucose testing. As a first impression, patients appear to quickly accept and welcome eAG. With eAG, patients will have a better understanding of glucose monitoring and can now correlate the results of HbA$_{1c}$ to eAG. Due to the added advantage that eAG brings to patient care, we expect that future consensus guidelines will recommend that laboratories report calculated eAG levels along with A$_{1c}$ values.

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Table 1. The relationship between glycated hemoglobin and estimated average glucose.

A$_{1c}$ (%)	eAG (mg/dL)
6	126
6.5	140
7	154
7.5	169
8	183
8.5	197
9	212
9.5	226
10	240

A$_{1c}$: Glycated hemoglobin; eAG: estimated average glucose; Formula utilized: eAG=28.7×A$_{1c}$-46.7

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A case of atrial fibrillation due to *Momordica charantia* (bitter melon)

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To the Editor: Eighty per cent of the population in developing countries continues to use traditional medicine for primary medical problems. *Momordica charantia* (bitter melon), belonging to the family Cucurbitaceae, has been frequently used as a medicine. In Turkish folk medicine, mature fruits of *Momordica charantia* are used externally for wound healing and orally for the treatment of peptic ulcer.[1] *Momordica charantia* has been credited with antidiabetic, antiseptic, antioxidant, anti-inflammatory, hypocholesterolemic, hypotensive, and immunostimulant properties.[2]

A 22 year-old man was admitted to our emergency department with complaints of palpitation and weakness. He had begun to use *Momordica charantia* two days before his admission for his dyspeptic complaints. He had crushed *Momordica charantia* and drunk two tablespoons of its juice three times a day, and had also drunk *Momordica charantia* juice on the morning of admission. The patient had no medical history of smoking, alcohol use, surgery, palpitation, coronary arterial disease, hypertension, diabetes, or chronic bronchitis. Physical examination revealed an arterial blood pressure of 100/70 mm Hg. The patient had a normal complete blood count, arterial blood gases, blood electrolyte (Na, K, Mg, Ca), and serial cardiac marker levels. Thyroxin and triiodothyronine concentrations and the findings of thyrotropin-releasing hormone tests were normal. Electrocardiography (ECG) showed atrial fibrillation (AF) with rapid ventricular response, approximately 136 beats/min (Figure 1a). Intravenous metoprolol was given to decrease the heart rate and intravenous amiodarone was administered for medical cardioversion

We administered intravenous amiodarone for medical cardioversion and sinus rhythm was restored after ten hours of infusion (Figure 1b). Because of AF lasted few than 2 days, anticoagulation therapy wasn't given to the patient. During continuous ECG monitoring in the emergency department, no recurrence of arrhythmia was observed, and transthoracic echocardiography found no structural or functional anomaly. Finally, the use of *Momordica charantia* was the only etiology that could explain the episode of AF. Hence, termination of the use of MC was recommended and the patient was discharged without any medication. The Naranjo criteria classify the probability that an adverse event is related to drug therapy based on a list of weighted questions, which examine factors such as the temporal association of drug administration and event occurrence, alternative causes for the event, drug levels, dose – response relationships and previous patient experience with the medication.[3] The Naranjo adverse drug reaction is assigned to a probability category from the total score as follows: *definite* if the overall score is 9 or greater, *probable* for a score of 5-8, possible for 1-4 and *doubtful* if the score is 0. The patient had six points according to the Naranjo

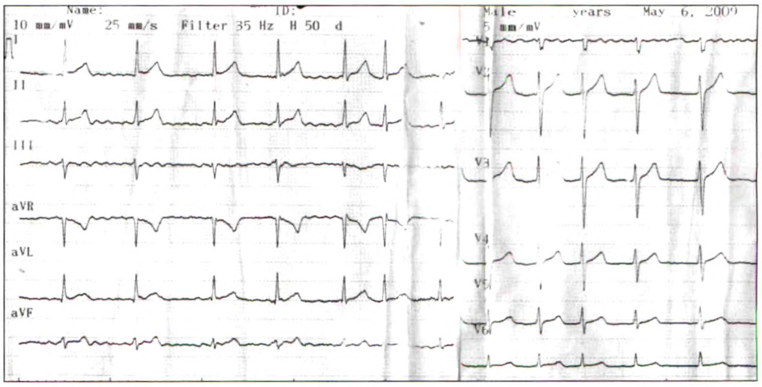

Figure 1a. Electrocardiography showed atrial fibrillation with rapid ventricular response.

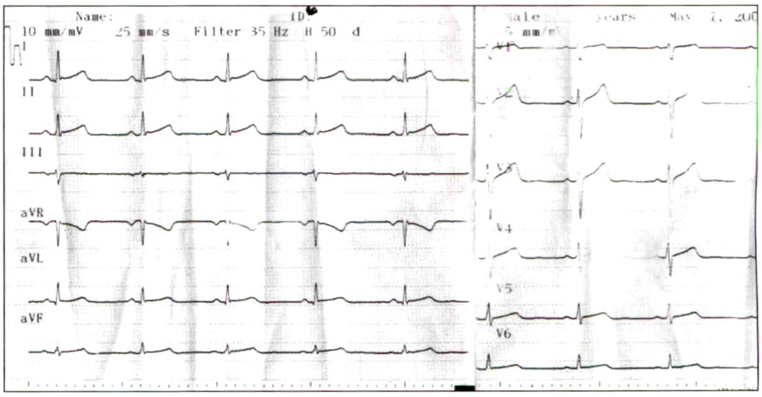

Figure 1b. Electrocardiography after cardioversion: sinus rhythm.

adverse drug reaction scale, which indicated a probable casual association. At the three-month followup visit, ECG showed normal sinus rhythm, and the patient reported no cardiac symptoms during the time until the visit.

Momordica charantia has been shown to ameliorate diet-induced obesity and insulin resistance, and has been reported to have beneficial effects such as lowered plasma lipid and blood sugar levels in animal studies.[2] *Momordica charantia* contains biologically active chemicals that include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins, and steroids. Several phytochemicals such as momorcharins, cryptoxanthin, cucurbitins, cycloartenols, elaeostearic acids, erythrodiol, galacturonic acids, goyasaponins, and multiflorenol have also been isolated from this plant.[4]

Momordica charantia has been shown to be safe in humans at a dose of 20 mg/kg body weight.[5] It has not been shown to be associated with nephrotoxicity, hepatotoxicity, or any adverse influence on food intake, growth organ weights, or hematological parameters. However, toxicity and even death have been reported in laboratory animals when high doses of the extracts were administered intravenously or intraperitoneally. The fruits and seeds have demonstrated greater toxicity than the leaves or aerial parts of the plant. Some documented adverse effects of *Momordica charantia* are hypoglycemic coma and convulsions in children, reduced fertility in mice, a favism-like syndrome, increases in gamma-glutamyltransferase and alkaline phosphatase levels in animals, and headaches.[2]

AF is the most common type of arrhythmia in adults. Cardiac conditions associated with the development of AF are hypertension, rheumatic mitral valve disease, coronary artery disease, and congestive heart failure. Noncardiac causes include hyperthyroidism, hypoxic pulmonary conditions, surgery, and alcohol intoxication.[6] We carefully ruled out cardiac and noncardiac organic pathologies that could have caused paroxysmal AF in this patient. The patient's palpitations developed in the 48 hours before his admission and the patient had no history of AF. Sinus rhythm was restored in the patient after the administration of antiarrhythmic medication. ECG showed normal sinus rhythm after three months of follow-up. As no cardiovascular rhythm disorders have been reported due to ingestion of bitter melon seeds, our case is noteworthy because it draws attention to the possibility that MC use can cause paroxysmal AF.

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Reactive macrophage activation syndrome in a patient with parvovirus B19 infection, lymphocytic lichenoid vasculitis, urticaria and angioedema

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A 47-year-old woman had a history of episodic acute intermittent angioedema and urticaria with moderate pruritus for one month. She was on 10 mg of loratadine daily. She had a fever of 39°C, arthralgia, fatigue, and angioedema of the upper respiratory tract. Laboratory results are shown in Table 1. She had elevated IgE (206 kU/L). The immunoassay for C1-esterase inhibitor was normal. She was treated with H1- and H2-blocking antihistamines, and methylprednisolone intravenously (1 mg/kg/day). Culture results and viral titers were negative except for a high positive titer of specific IgG antibody to parvovirus B19 of 11.1 (positive titer >1). On the third week of hospitalization, she deteriorated rapidly and developed a macular rash on the trunk and extremities with generalised lymphadenpathy, liver dysfunction and disseminated intravascular coagulopathy (DIC) (Figure 1). A skin biopsy specimen was compatible with lymphocytic lichenoid vasculitis. An inflammatory pattern centered on the basal layer of the epidermis and upper dermis in a dense band-like distribution. Direct immunofluorescence showed no IgG, IgA, IgM, C3, C1q and fibrinogen deposits. A bone marrow aspirate showed hemophagocytosis (Figure 2). Parvovirus B19 DNA was detected by the polymerase chain reaction (PCR) in bone marrow (Figure 3). Macrophage activation syndrome was confirmed. The patient was treated with methylprednisolone 250 mg/day intravenously and intravenous immunoglobulin (IVIG) 0.55 g/kg BW/day for five consecutive days, followed by methylprednisolone 1 mg/kg daily. Fresh frozen plasma and enoxaparin were administered. Two days after treatment, she improved. Monthly infusions of IVIG were continued for 6 months. Corticosteroids were tapered gradually to 8 mg of methylprednisolone daily. On periodic follow-up, the patient was quite well without episodes of angioedema and no new skin lesions were seen. Our patient met all criteria for reactive macrophage activation syndrome (rMAS) outlined by Imashuku.[1]

Acute parvovirus B19 infection can be diagnosed by demonstrating a four-fold rise in serum B19-specific IgG antibody titers, as in our case. B19 specific DNA or antigens can be detected for months or even years after infection.[2,3] We speculate that the presence

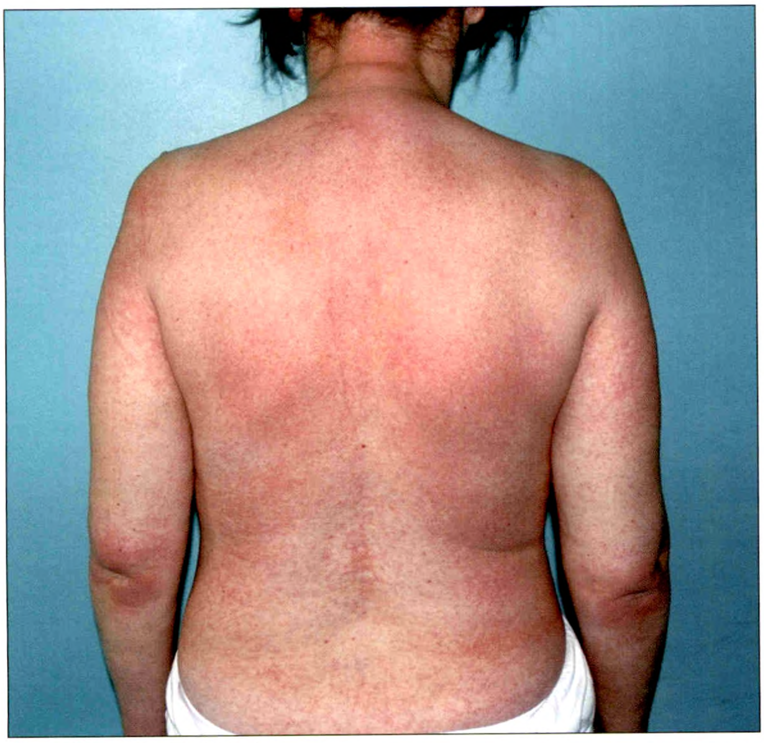

Figure 1. Maculopapular rash.

Table 1. Laboratory test results.

Laboratory data (normal values in parenthesis)	Admission	Before IVIG[a]	After first IVIG[a]
Erythrocyte sedimentation rate (2-13 mm/h)	57	16	122
White blood cell count (4-10×10^9/L)	11.8	5.8	11.0
Hemoglobin (120-160 g/L)	132	78	101
Platelet count (140-440×10^9/L)	203	33	249
C reactive protein (0-5 mg/L)	143	88.2	28.7
Aspartate aminotransferase (7-49 IU/L)	28	720	54
Alanine aminotransferase (7-49 IU/L)	35	500	75
Lactate dehydrogenase (170-430 IU/L)	361	3206	335
Ferritin (10-300 mg/L)	12200	43700	8300
Fibrinogen (2-4.5 g/L)	6.1	1.4	4.0
D-dimer (0.06-0.25 mg/ml)	0.15	4.36	0.30

[a] IVIG = intravenous immunoglobulin

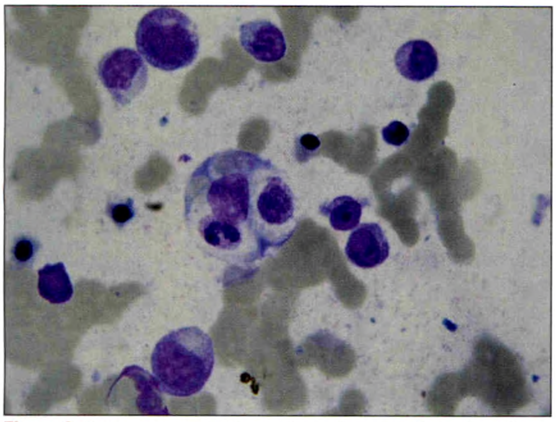

Figure 2. Bone marrow aspirate shows increased number of histiocytes with active hemophagocytosis (Hematoxylin stain ×200 magnification).

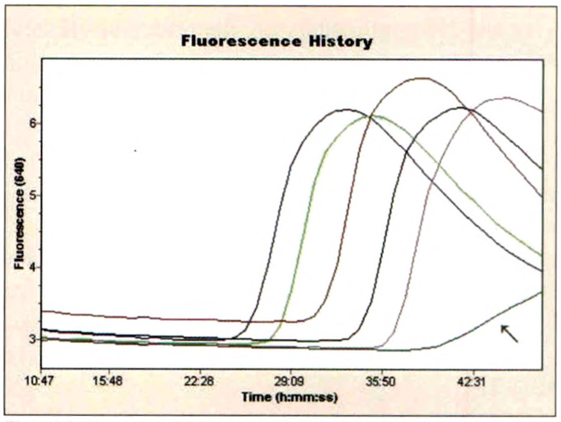

Figure 3. Real-time PCR that confirmed parvovirus B19 (arrow shows negative control sample).

of acute parvovirus B19 infection was a trigger for rMAS.[4] Lymphocytic vasculitis is a reactive process.[5,6] Purpura pigmentosa chronica, fixed drug eruption, urticarial vasculitis, allergic vasculitis, and the vasculitis of Sjogren syndrome are known to have lymphocytic vasculitis. We conclude that rMAS might represent a subgroup of patients with systemic inflammatory response amenable to IVIG treatment. Given early, IVIG may interrupt the processes that lead to macrophage overactivation.

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Good reporting of trials and observational studies: Related guidelines and initiatives

EQUATOR

EQUATOR **<http://www.equator-network.org>** (Enhancing the QUAlity of Trials and Other Research) The EQUATOR Network is a new initiative that seeks to improve the quality of scientific publications by promoting transparent and accurate reporting of health research.

STROBE

STROBE **(STrengthening the Reporting of OBservational studies in Epidemiology)** is an international, collaborative initiative of epidemiologists, methodologists, statisticians, researchers and editors involved in the conduct and dissemination of observational studies.

CONSORT (Consolidated Standards of Reporting Trials), is part of a broader push to produce reporting guidelines (RGs) for many different types of research. The main product of CONSORT is the CONSORT Statement, which is an evidence-based, minimum set of recommendations for reporting RCTs. It offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation. The CONSORT Statement comprises a 22-item checklist and a flow diagram, along with some brief descriptive text.

ASSERT

The ASSERT **(A Standard for the Scientific and Ethical Review of Trials)** initiative is a proposed standard for the review and monitoring of randomized clinical trials by research ethics committees. Among other items, its checklist incorporates certain elements of CONSORT, to ensure fulfillment of the requirements for scientific validity in the ethical conduct of clinical research.

MOOSE

The MOOSE **(Meta-analysis of Observational Studies in Epidemiology)** paper reports the outcome of a 1997 workshop to examine the reporting of meta-analyses of observational studies. It makes recommendations to aid authors, editors, reviewers and readers of observational research.

QUOROM

The QUOROM **(Quality Of Reporting Of Meta-analyses)** conference resulted in the creation of the QUOROM Statement, which consists of a checklist and flow diagram.

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The STARD **(Standards for Reporting of Diagnostic Accuracy)** initiative aims to improve the accuracy and completeness of reporting of studies of diagnostic accuracy in order to allow readers to assess the potential for bias in a study and to evaluate the generalisability of its results.

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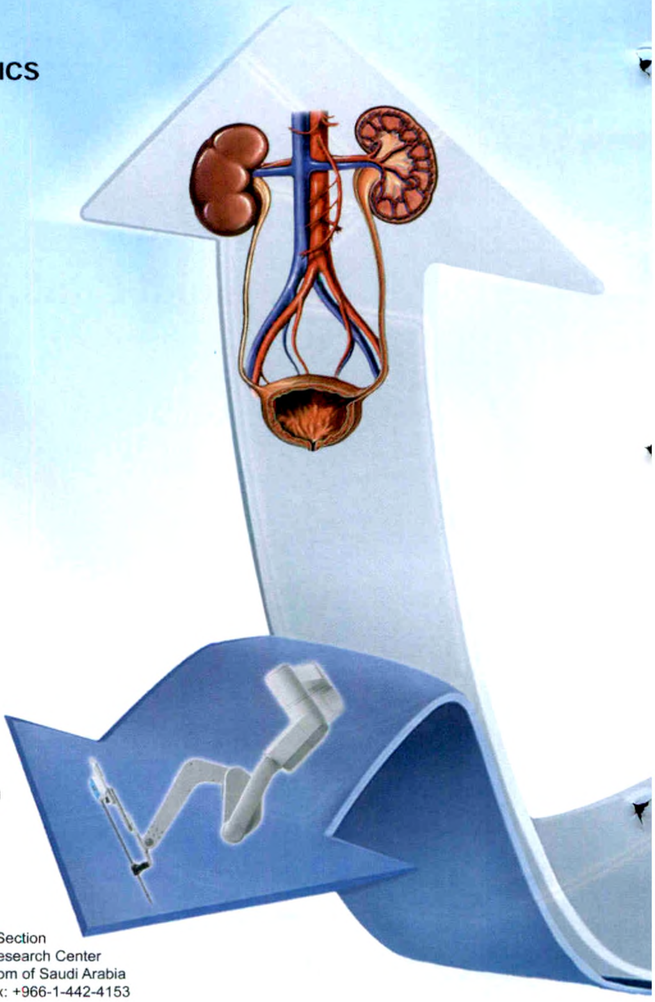

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جين Glu298Asp الأنزيم المشكل لأكسيد النتريك البطاني متعدد الأشكال عامل خطر مستقل في مرض الشريان الإكليلي بإيران

مقدمة: يتم تركيب أكسيد النتريك NO في بطانة الأوعية من L-أرجينين بواسطة أنزيم تركيب أكسيد النتريك البطاني NOS3. وبما أن نقص تركيب NO في الخلايا البطانية مترابط بتطور التصلب العصيدي الإكليلي، فقد قمنا بالاستقصاء حول ارتباط تعدد أشكال جين NOS3 بمرض الشريان الإكليلي CAD لدى السكان الإيرانيين.

المرضى والطرق: قمنا بإجراء دراسة تعدد أشكال جين Glu298Asp الأنزيم المركب لأكسيد النتريك البطاني على ٢٤١ حالة مصابة بمرض الشريان الإكليلي، مع إيجابية الصور الوعائية (تضيق إلى أقل من ٥٠٪ في وعاء دموي واحد على الأقل) في مستشفى "شاهد راجي" للقلب و ٢٦١ حالة شاهدة ممن ليس لديهم تاريخ طبي يشير إلى معاناتهم من أعراض مرض الشريان الإكليلي. وتم تحليل تعداد أشكال جين NOS3 من خلال تحديد تفاعل البلمرة التسلسلي PCR وتفاوت أطوال الشدف المقطعة RFLP وفحص الدهون وعوامل خطر أخرى أيضاً.

النتائج: كانت تواترات النمط الجيني لتعدد أشكال Glu298Asp بالنسبة للـ Glu/Glu,Glu/Asp و Asp/Asp ٦١,٣٪ و ٣٢,٢٪ و ٦,٥٪ في الحالات الشاهدة، و ٤٦,٥٪ و ٤٢,٧٪ و ١٠,٨٪ لدى المصابين بمرض الشريان الإكليلي، على التوالي. وقد اختلفت مؤشرات النمط الجيني بشكل واضح بين المجموعتين (P=٠.٠٠٣). وبلغت مؤشرات الألائل ٣٢,٢٪ و ٢٢,٦٪ لدى الحالات التي تعاني من مرض الشريان الإكليلي والحالات الشاهدة، على التوالي، وكانت مختلفة بشكل واضح بين المجموعتين (P=٠,٠٠١ نسبة الاحتمال =١,٦). كما ارتفع معدل دهون البلازما ماعدا HDL-C بشكل واضح لدى المجموعة المصابة بمرض الشريان الإكليلي.

الاستنتاج: تشير هذه النتائج إلى ترابط مرض الشريان الإكليلي بتعدد الشكل Glu298Asp في جين الأنزيم المركب لأكسيد النتريك البطاني لدى سكاننا ويعد هذا التعدد عامل خطر مستقل لمرض الشريان الإكليلي.

انتشار زيادة ناقلة الأمين الكبدية لدى المرضى الأردنيين الذين يعانون من النوع الثاني من داء السكري

مقدمة: تهدف هذه الدراسة إلى تقدير معدلات انتشار المستويات المرتفعة لناقلة الأمين الكبدية لدى مرضى النوع الثاني من داء السكري وتحديد العوامل المصاحبة.

المرضى والطرق: أجريت هذه الدراسة على ١٠١٤ مريضاً من مرضى النوع الثاني من داء السكري ١ الذين راجعوا العيادات الخارجية بصفة متعاقبة، وحضروا إلى المركز الوطني لداء السكري والغدد الصماء والوراثة في الأردن. وقد تم تدوين العمر والجنس ومنسب كتلة الجسد ومحيط الخصر ومستويات الشحوم وناقلة الأمين الكبدية. كما أجري تصوير بالأمواج فوق الصوتية للمرضى الذين أظهروا ارتفاعاً في مستويات ناقلة الأمين الألانينية.

النتائج: بلغ معدل انتشار ناقلة الأمين الألانينية، إجمالاً ١٠،٤٪ (العدد ١٠٥) وكان معدل الانتشار المرتبط بالجنس ١٢،٨ العدد (٧١) عند الرجال و٧،٤ (العدد ٣٤) عند النساء. كان معدل انتشار ناقلة الأمين الأسبرتية ٥،٤ (العدد ٥٥) وكان معدل الانتشار بالنسبة للجنس ٦،٠٪ (العدد ٣٣) ٢ عند الرجال و ٥،٢٪ (العدد ٢٤) عند النساء. وقد ظهرت المستويات المرتفعة لكل من ناقلة الأمين الألانينية وناقلة الأمين الأسبرتية عند ٤،٥٪ (العدد ٤٤) من المرضى فقط. عند الذكور (نسبة الأرجحية = ٢،٣٥، نطاق الثقة: ١،٥-٣،٨) مع ارتفاع محيط الخصر، (صاحب نسبة الأرجحية = ١،٩، نطاق الثقة: ١،٢-٣،٢ خطرا متزايداً لإرتفاع مستويات ناقلة الأمين الألانينية. كان لدى المرضى الأصغر سناً ميلاً أعلى لإرتفاع ناقلة الأمين الألانينية مقارنة بالمرضى الذين تجاوزا سن ٦٥ عاماً (نسبة الأرجحية= ١٢،٤ للفئة العمرية ٢٥ • ٤٥ عاماً و نسبة الأرجحية = ٥،٨ للمرضى الذين تراوحت أعمارهم بين ٤٥ و٦٥ سنة). وكان عدم استخدام الأنسولين مصحوباً بارتفاعات كبيرة ٤ لمستويات ناقلة الأمين الألانينية (نسبة الأرجحية =١،٧ CI: ١،١- ٢،٩).

الاستنتاج: ظهرت المستويات المرتفعة لناقلة الأمين الألانينية وناقلة الأمين الأسبرتية عند ١٠،٤ و٥،٤٪ من مرضى النوع الثاني من مرض السكري على التوالي. شكل الجنس الذكوري، وصغر السن، وارتفاع محيط الخصر كمؤشر على السمنة المركزية، إلى جانب عدم استخدام الأنسولين عوامل تكهن مستقلة على ارتفاع مستويات ناقلة الأمين الكبدية.

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دراسة مراجعة تحليلية حول المضاعفات التي يعاني منها المتبرعون الأحياء بالفص الكبدي الأيمن

مقدمة: تتزايد أعداد البالغين المتلقين لطعم كبدي من الفص الكبدي الأيمن في عمليات زراعة الكبد من متبرع حي. ومع ذلك فإن إصابة المتبرعين أنفسهم بمضاعفات هي أكثر تواردا ً منها في تطعيمات الفص الأيسر. وعلى ذلك قمنا بتحليل المضاعفات الناجمة بعد العملية لدى المتبرعين بالفص الكبدي الأيمن كخطوة لتحسين نتائج المتبرعين.

المرضى والطرق: قمنا بتحليل المعلومات حول المضاعفات التالية للعملية لدى المتبرعين بالفص الكبدي الأيمن من خلال ٣١١ عملية زراعة كبد تم إجراؤها من فترة فبراير ١٩٩٨م إلى ديسمبر ٢٠٠٣م.

النتائج: تراوحت أعمار المتبرعين من ١٩ إلى ٦٤ سنة (متوسط: ٤٦ سنة). وتراوح منسب كتلة الجسم لديهم من ١٦,٦ إلى ٣٤,٣ (متوسط: ٢٢,١). وكان المتوسط المعياري لمدة العملية ٦,٥٨ (١,٢٥) ساعة، وفقدان الدم ٢٨٩ (٢٥٤) مللتر. بلغ متوسط حجم الجزء المتبقي من الكبد لدى المتبرع ٤٢,٢ ٪ (معدل: ٢٠,٦-٦٠,٣٪) ومتوسط الإقامة في المستشفى ١٤,٥ يوماً (معدل: ٦-٢٦٧). وقد توفي متبرع واحد بسبب قصور كبدي ناجم عن صغر حجم الجزء المتبقي من الكبد (٢٦٪) و الإصابة بالتهاب الكبد الشحمي. كما أصيب ثلاثون بالمئة من المتبرعين بواحد أو أكثر من المضاعفات، حدثت ١٢٢ (٣٣,٤٪) من المضاعفات لدى ١٢٣ متبرع. ووفقاً لتصنيف "كلافيين' Clavien أصيب بمضاعفات الدرجة الأولى ٧١ متبرع (٥٧,٧٪)، و بمضاعفات الدرجة الثانية ٩ متبرعين (٧,٣٪)، و بمضاعفات الدرجة الثالثة أ ٣٩ متبرع (٣١,٧٪)، و بمضاعفات الدرجة الثالثة ب ٣ متبرعين (٢,٥٪) . وبمضاعفات الدرجة الخامسة متبرع واحد (٠,٨٪) وكانت مضاعفات الصفراء الأكثر شيوعاً (١٢,٨٪)، يليها بقايا تجمع السوائل البطنية (١٢٪).

الاستنتاج: إن التبرع بالفص الكبدي الأيمن إجراء يحظى بقبول واسع مما يؤدي إلى زيادة الإدراك لدلالة عملية زراعة الكبد من متبرع حي لدى شريحة كبيرة من المتبرعين. من جهة أخرى، فإن حجم الجزء المتبقي من الكبد والتغيرات التشريحية في المسار الصفراوي يعتبر من أهم عوامل الخطر بالنسبة للمضاعفات التالية لعملية زراعة الكبد.

انتقال فيروس نقص المناعة البشري (HIV) من الأم إلى الطفل: تجربة في مستشفى إحالة في السعودية

مقدمة: تم الإبلاغ سابقاً على أن معدل انتقال فيروس نقص المناعة البشري (HIV) من النوع (١) من الأم إلى الطفل كان مرتفعاً في السعودية. وهذا المعدل كان بين مجموعة من النساء المصابات بفيروس نقص المناعة البشري (HIV) والملتحقات ببرنامج وقائي مسيطر عليه بشكل جيد في منشأة للرعاية الطبية المتخصصة بالرياض.

المرضى والطرق: شملت الدراسة جميع النساء المصابات بفيروس نقص المناعة البشري (HIV) اللاتي حملن وولدن خلال متابعاتهن بين شهر يناير من ١٩٩٤م وشهر يونيو من عام ٢٠٠٦ م. وقد تم تسجيل كل من العبء الفيروسي وCD4 وتعداد اللمفاويات التائية وموعد الولادة وطريقتها بالإضافة إلى حالة فيروس نقص المناعة البشري (HIV) في المولود عند عمر ١٨ شهراً. كما تم تقديم الإرشاد لجميع النسوة بالإضافة إلى تلقي العلاج وفقاً لبروتوكول PACTG ٠٧٦ ذي الخطوات الثلاثة.

النتائج: من بين ٦٨ من النساء المصابات بفيروس نقص المناعة البشري (HIV)، ٣١ حملت منهن ما مجموعه ٤٠ حملاً. وقد أجهضت إحداهن في ١٣ أسبوعاً من الحمل. أما طريقة الولادة فكانت ولادة قيصرية اختيارية في ٢٨ من حالات الحمل (٧٠٪) وذلك في الأسبوع ٣٦ من الحمل. و ١١ (٢٧,٥٪) أتممن ولادة مهبلية طبيعية. وكان متوسط تعداد CD4 وتعداد اللمفاويات التائية إبان خلية/ميكرولتر الولادة ٥٣٦ وكان متوسط العبء الفيروسي إبان الولادة لـ ٢٥ من حالات الحمل (٧٥٪) ١٦٤٦ نسخة/مل. مع ٩ من حالات الحمل فقط (٢٢,٥٪) التي كان العبء الفيروسي فيها أكثر من ١٠٠٠ نسخة/مل. لم يتم الكشف عن فيروس نقص المناعة البشري (HIV) قبل الولادة في ١٤ من حالات الحمل (٣٥٪). كانت جميع المريضات قيد العلاج المضاد للفيروس أثناء فترة الحمل والولادة. كانت نتيجة تحليل فيروس نقص المناعة البشري (HIV) في كل من المواليد عند عمر ١٨ شهراً، والذي يبلغ عددهم ٣٩ . سلبية. وجدير بالذكر، أن المواليد لم يتم إرضاعهم طبيعياً.

الاستنتاج: على عكس الخبرة المحلية السابقة والتشخيص والعلاج. فقد منع العلاج المضاد للفيروس تقريباً انتقال فيروس نقص المناعة البشري (HIV) من النوع (١) من الأم إلى الطفل بين سكاننا. ٢

أنفلونزا A H١N١ في المملكة العربية السعودية: وصف لأول مائة حالة

مقدمة: أعلنت منظمة الصحة العالمية في أبريل ٢٠٠٩م أن أنفلونزا H١N١ “ من الطوارئ الصحية على الصعيد العالمي'. وفي ١١ يونيو ٢٠٠٩م رفعت المنظمة تحذير الوباء إلى المستوى ٦ (إشارة إلى أنها جائحة عالمية). وبحلول أغسطس ٢٠٠٩م أعلنت أكثر من ١٧٠ دولة ومقاطعة عن حالات إصابة بأنفلونزا الخنازير. تم تلخيص الوصف الوبائي لأول ١٠٠ حالة مصابة بالفيروس في المملكة العربية السعودية في هذا التقرير.

المرضى والطرق: تم جمع البيانات عن طريق استبيان مصمم مسبقاً بين ١ يونيو إلى ٣ يوليو ٢٠٠٩م. قام برنامج الوبائيات الحقلي بإكمال الاستبيان وتصنيف وتحليل بيانات أول ١٠٠ حالة أثبتت إصابتها بأنفلونزا A (H١N١) باستخدام برنامج معلومات الوبائيات.

النتائج: تتراوح أعمار الحالات المبلّغ عنها بين ١ إلى ٥٦ عاماً، وكانت أعلى نسبة عمرية بين ٢٠ إلى ٣٠ عاماً تلتها المجموعة العمرية ١ إلى ١٠ أعوام. وكانت نسبة الإناث ٥٥٪ من الحالات. أما الحالات القادمة من الخارج فقد كانت بنسبة ٤٧٪. وأكثر الجنسيات المصابة السعودية والفلبينية. شملت الأعراض الرئيسية الحمى (٥٦٪) والسعال (٥٤٪) والتهاب الحلق (٣٢٪). ووصل عدد الحالات إلى ذروته بين ٢٧ • ٢٩ يونيو في مطار الملك خالد الدولي حيث وصلت نسبة الحالات المصابة من الخارج إلى ٥٨٪.

الاستنتاج: ما يزال أنفلونزا H١N١ يهدد المملكة العربية السعودية، ويجب تطبيق معايير شاملة وفعالة لمراقبة المرض والوقاية منه لنتمكن من السيطرة على انتشاره. وستعود فوائد إتباع هذه المعايير على جميع الدول التي ترسل الحجاج لأداء فريضة الحج.

الخلاصات العربية

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تصدر المدونات الطبية السعودية مرة كل شهرين عن مستشفى الملك فيصل التخصصي ومركز الأبحاث ، ص.ب ٣٣٥٤ ، الرياض ١١٢١١، المملكة العربية السعودية. يتم توزيع المدونات وإعداد الملخصات العربية بالتعاون مع د. زهير القاوي بواسطة موظفي إدارة الخدمات الإدارية بمستشفى الملك فيصل التخصّصي ومركز الأبحاث. جميع المراسلات والاستفسارات وطلبات الاشتراك ترسل إلى المحرر الأول مكتب المطبوعات (ر.إ ٣٦).

سعر الاشتراك ١٠٠ ريال في السنة للأفراد داخل المملكة و٢٠٠ ريال في السنة للأفراد خارج المملكة و٥٠٠ ريال في السنة للمؤسسات. يمكن فتح اشتراك مجاني للأفراد و المؤسسات في الدول النامية.

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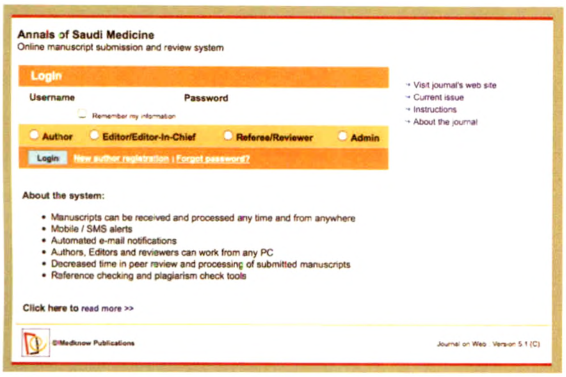
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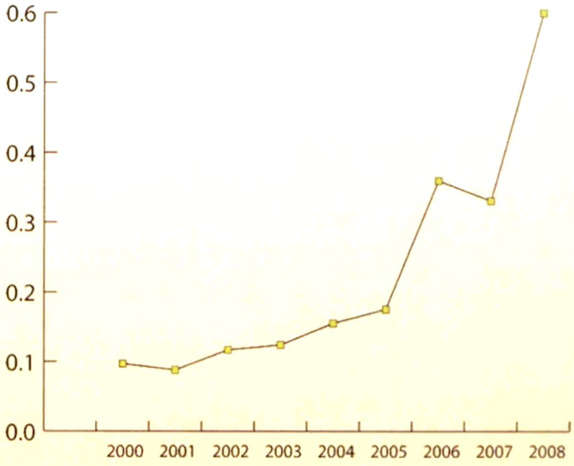

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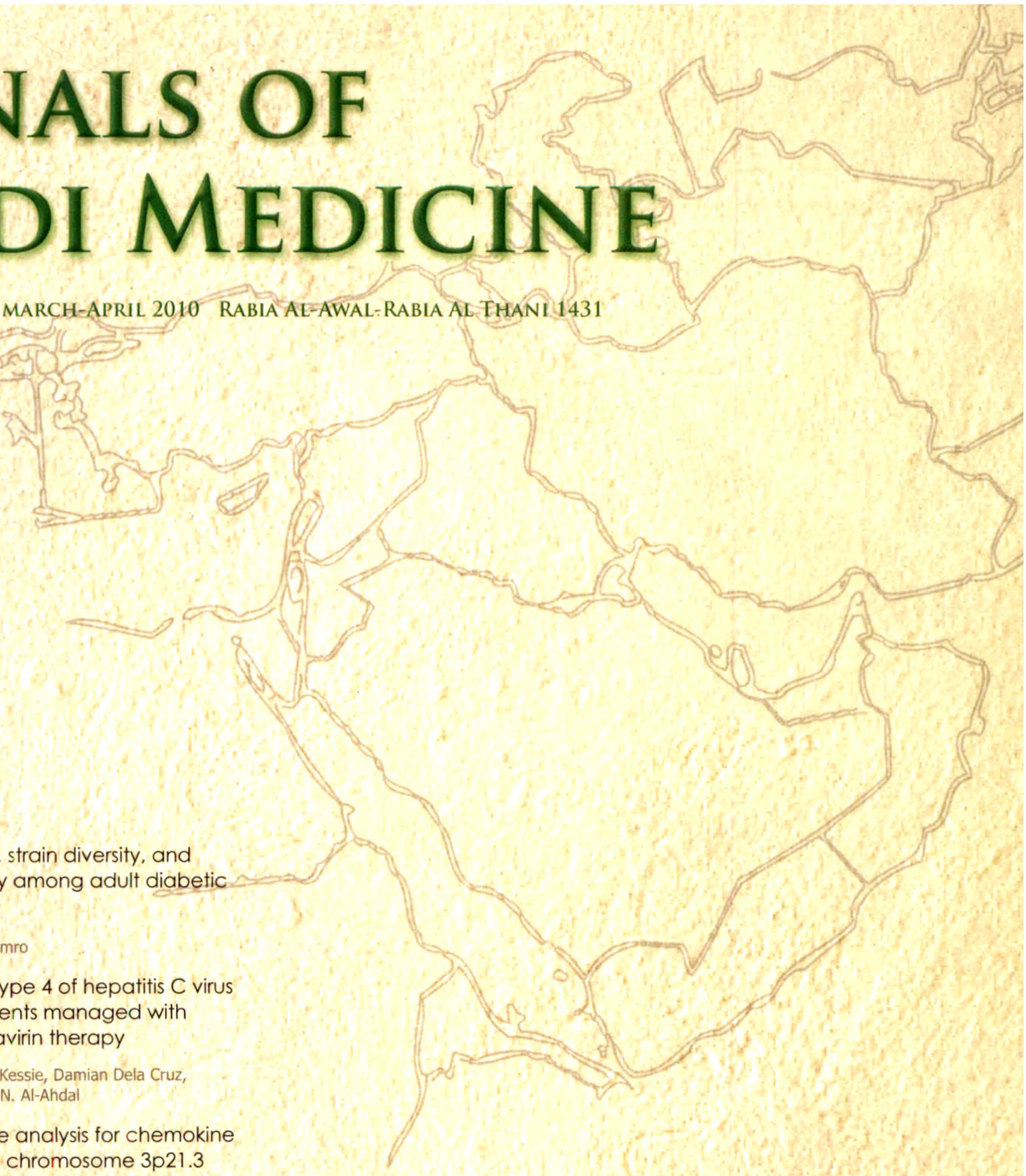

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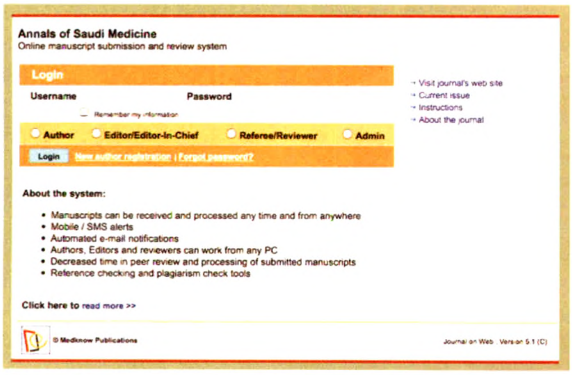
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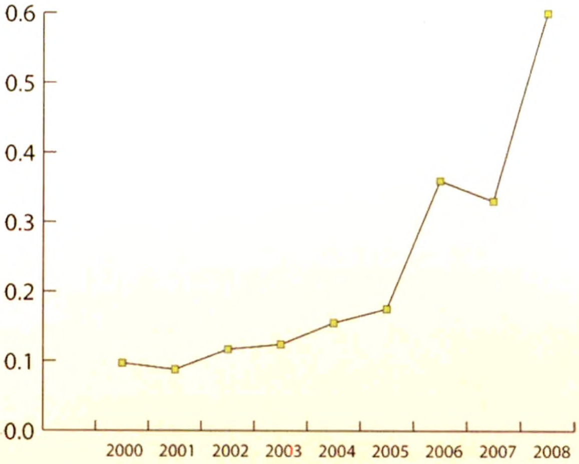

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20 January 2010 / 05 Safar 1431
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MARCH (6)

2nd International Conference on "Radiation Medicine: Innovative Approaches & Interprofessional Practice"
01-04 March 2010 / 15-18 Rabi Al Awal 1431
Contact No.: 4427879 (Biomedical Physics, Research Center)

1st Middle East Symposium on Diseases of the Esophagus
09-10 March 2010 / 23-24 Rabi Al Awal 1431
Contact No.: 4647272 Ext. 32958 (Surgery)

22nd Saudi Urological Conference
15-18 March 2010 / 29 Rabi I – 02 Rabi II 1431
Contact No. 4647272 Ext. 31830 or 4424302 (Urology)

1st International Conference in Pediatric Emergencies, Injury & Disability Prevention
23-25 March 2010 / 07-09 Rabi Al Thani 1431
Contact No.: 4424425 (Emergency Medicine)

Annual Saudi Colorectal Surgery Forum
27-31 March 2010 / 11-15 Rabi Al Thani 1431
Contact No.: 4427754 (Surgery)

3rd Mini AABB Symposium
30 March – 01 April 2010 / 14-16 Rabi Al Thani 1431
Contact No.: 4424224 (Pathology & Laboratory Medicine)

APRIL (3)

1st Annual Pediatric Nephrology Meeting
06-07 April 2010 / 21-22 Rabi Al Thani 1431
Contact No.: 4427798 (Pediatrics)

Medication Safety: A Mission for All Symposium
13-14 April 2010 / 28-29 Rabi Al Thani 1431
Contact No.: 4427601(Pharmacy Services Division)

International Symposium on New Frontiers in Breast Cancer
27-29 April 2010 / 13-15 Jumada Al Awal 1431
Contact No. 4647272 Ext. 31830 or 4423940 (Oncology Center)

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Conference on Diagnostic & Prognostic Testing in Clinical Biochemistry – A New Age of Disease Biomarkers
04-05 May 2010 / 20-21 Jumada Al Awal 1431
Contact No.: 4427224 (Pathology & Laboratory Medicine)

Oncology Centre Academic & Research Day
05 May 2010 / 21 Jumada Al Awal 1431
Contact No.: 4647272 Ext 38005 Oncology Center

Preventative Genetics: New Initiatives Workshop
11-12 May 2010 / 27-28 Jumada Al Awal 1431
Contact No.: 4424988 (Medical Genetics)

12th Clinical Research Professionals' Course
15-26 May 2010 / 01-12 Jumada Al Thani 1431
Contact No.: 4424987
(Centre for Clinical Studies and Empirical Ethics)

Special Care Dentistry Symposium
18-19 May 2010 / 04-05 Jumada Al Thani 1431
(Workshop on 20 May 2010 / 06 Jumada Al Thani 1431)
Contact No. 4647272 Ext. 31830 or 4427652 (Dentistry)

Ultrasound Guided Regional Anesthesia & Vascular Access Workshop
26-27 May 2010 / 11-12 Jumada Al Thani 1431
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8th Annual Academic Day
26 May 2010 / 12 Jumada Al Thani 1431
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3rd Pediatric Infectious Diseases Seminar
01-02 June 2010 / 18-19 Jumada Al Thani 1431
Contact No.: 4427761 (Pediatrics)

5th LC & LCMS: Concept & Hands-On Training
05-09 June 2010 / 22-26 Jumada Al Thani 1431
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26-28 October 2010 / 18-20 Dhu Al Qada 1431
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28 October 2010 / 20 Dhu Al Qada 1431
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SUMMARIES

INFECTIOUS DISEASE

Candidal colonization, strain diversity, and antifungal susceptibility among adult diabetic patients

In a case-control study of 150 diabetics vs 50 healthy controls, Al-Attas and Oda found that diabetics had a higher candidal carriage rate, but not density. None of the salivary samples from controls were resistant to any tested antifungal, while diabetic samples showed varying resistance to azole antifungals. There was a significant positive correlation between glycemic control and colonization in type 2 diabetics. Glycemic control and salivary pH may be important in controlling candidal infections.

Quasispecies of genotype 4 of hepatitis C virus genomes in Saudi patients managed with interferon alfa and ribavirin therapy

In an attempt to determine whether quasispecies population might predict response to therapy, Al-Qahtani and colleagues studied the quasispecies population of HCV genotype 4 in 25 naive patients at the start of interferon alfa and ribavirin combination therapy and at 3 and 6 months later. Quasispecies could not predict response to therapy in the population studied. Patients with stable viral populations or viral populations that decreased in complexity following treatment tended to respond to therapy.

ASTHMA AND ATOPY

Linkage and haplotype analysis for chemokine receptors clustered on chromosome 3p21.3 and transmitted in family pedigrees with asthma and atopy

Al-Abdulhadi and Al-Rabia genotyped 150 nuclear families with at least two children or young adults with a physician diagnosis of asthma and current symptoms. The linkage and haplotype analyses confirmed that three mutations were linked with asthma but not with atopy. The results suggest an association between chemokine receptor genes clustered on chromosome 3 and asthma, but further studies are needed to confirm the association.

ONCOLOGY

A meta-analysis of highly active anti-retroviral therapy for treatment of plasmablastic lymphoma

A meta-analysis by Guan and colleagues showed that the prognosis for plasmblastic lymphoma was improved in patients receiving HAART in addition to chemotherapy and/or radiotherapy than in patients receiving chemotherapy and/or radiotherapy alone, but the small sample sizes in the eight individual studies raise the likelihood of bias. The results should be taken cautiously. Larger studies are needed to determine whether addition of HAART carries a survival advantage.

RADIOLOGY

Inter- and intraobserver variation between radiologists in the detection of abnormal parenchymal lung changes on high-resolution computed tomography

Al-Khawari and colleagues used the HRCT images from 65 patients with systemic lupus erythematosus with lung involvement to assess agreement between four radiologists in detecting abnormal parenchymal lung changes. There was a clinically acceptable level of agreement between all radiologists, but increased variability in agreement when a radiologist was not involved on a regular basis in thoracic imaging.

OBSTETRICS

Fetal weight normograms for singleton pregnancies in a Jordanian population

Al-Bayyari and Abu-Heija obtained estimated intrauterine fetal weights by ultrasonography from 600 pregnant women between 20 to 42 weeks of gestation and calculated percentiles by gestational age and sex for singleton pregnancies. Variables significantly associated with intrauterine weights were weight gain during pregnancy, maternal hemoglobin level, frequency of antenatal visits, smoking and fetal sex. Weight gain should be encouraged for mothers who gain less than one kilogram per month in the second and third trimester.

original article

Candidal colonization, strain diversity, and antifungal susceptibility among adult diabetic patients

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BACKGROUND AND OBJECTIVES: Candidal colonization in diabetics is a matter of debate. The aim of this study is to investigate oral candidal colonization, strain diversity, antifungal susceptibility, and the influence of local and systemic host factors on candidal colonization in adult diabetics.

METHODS: We conducted a case-control study that compared 150 diabetics (49 type 1, 101 type 2) with 50 healthy controls. Two salivary samples were collected, using the oral rinse sampling method: one for salivary flow rate and pH determination, and the other for candidal colonization assessment. The candidal isolates were identified and tested in vitro for antifungal susceptibility using the commercial kit, Candifast. The relationship between specific host factors and candidal colonization was also investigated.

RESULTS: Diabetics had a higher candidal carriage rate compared to controls, but not density. *Candida albicans* was the most frequently isolated species, but diabetics had a variety of other candidal species present. None of the control samples were resistant to any tested antifungal, while the diabetic samples had differing resistances to azole antifungals. Although there was a significant positive correlation between glycemic control and candidal colonization in type 2 diabetics, there was a negative correlation between salivary pH and candidal carriage in the controls versus density in type 2 diabetics.

CONCLUSIONS: Diabetic patients not only had a higher candidal carriage rate, but also a variety of candidal species that were resistant to azole antifungals. Oral candidal colonization was significantly associated with glycemic control, type of diabetes, and salivary pH.

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of candidal species, the most common being *Candida albicans*.[1] The underlying causes of oral candidiasis include extremes of age, xerostomia, antibiotic therapy, dentures, smoking, Cushing syndrome, malignancies, immune deficiencies, and diabetes mellitus (DM).[1,2] The presence of *Candida* species and the density of candidal growth in the oral cavity is often said to be increased in patients with DM.[3,4] However, these observations remain controversial.[5] Similarly, there are conflicting reports on the identity of the underlying risk factors.[3-5] These uncertainties are thought to be related to the different pathophysiological behaviors of the two clinical types of DM, to different patients and disease data such as, age, duration or control of diabetes or, at least in part, are thought to reflect racial and environmental differences among diabetic populations worldwide.

Diabetes mellitus, specifically type 2 diabetes, is a major public health issue. The diabetic population is expected to increase from 171 million in 2000 to 366 million by 2030.[6] World Health Organization statistics show that, worldwide, almost three million deaths per year are attributed to diabetes, equivalent to 5.2% of all deaths.[7] DM is emerging as a major public health problem in Saudi Arabia, parallel with the worldwide diabetes pandemic. Early reports indicated that nearly one Saudi in five of age 30 years or older had DM,[8] while the latest report showed that the overall prevalence of

DM among adult Saudis of both sexes in rural as well as urban communities is 23.7%.[9] Despite these recognized high rates of DM among the Saudi population, there is an obvious lack of published studies evaluating the prevalence and characteristics of oral fungal infections, or investigating the underlying risk factors associated with DM, in Saudi Arabia. The aim of this study is to investigate oral yeast colonization (rate and density of acquisition of *Candida* species colonies), strain diversity, and antifungal susceptibility in adult diabetics, and to evaluate the influence of some local and systemic host factors on candidal colonization.

METHODS

This case-control study was conducted on 150 diabetic patients (49 type 1, 101 type 2) and 50 healthy controls. The diabetic patients were recruited from the diabetic clinics of King Abdul-Aziz University Hospital (KAAUH), Jeddah, Saudi Arabia, during routine diabetic follow-up appointments. Consecutive eligible patients identified on a specific sampling day were asked to participate. The inclusion criteria were a diagnosis of either type 1 or 2 DM and age of 18 years or older. The control group included age-grouped and sex-matched healthy volunteers (companions of the diabetic patients as well as dental auxiliaries) with no history of diabetes, who were selected from KAAUH. All subjects in both groups gave a signed informed consent to participate in the study. Individuals who had received antibiotics or steroid therapy or had been using antiseptic mouthwash during the prior three weeks were excluded from the study. The study protocol was approved by the ethics committee of the KAAUH medical faculty.

A structured questionnaire was developed for collecting information on demographics (age and gender), medical variables (diabetes type, duration, and presence of diabetes-related systemic diseases), and local factors (denture status, oral hygiene, and smoking). The investigators supervised the completion of the questionnaire, which had close-ended questions including options. The patient's medical records were used to gather information on diabetes type and duration, as well as, the presence of diabetes-related systemic complications (retinopathy, nephropathy, neuropathy, and peripheral vascular diseases). The presence of any of these complications was considered a positive finding and dichotomized on a scale of yes or no. All subjects provided three samples, two salivary and one blood sample, which were sent immediately to the KAAUH Microbiology and Hematology Laboratories, respectively. To prevent circadian variations, the samples were collected between 9 a.m. and 12 noon.

The subject was typically instructed not to eat, drink or smoke two hours prior to sample collection. They were asked to lean their upper body forward, and allow oral fluid to drip into a graduated collection vial, over a five-minute period, without swallowing.[10] This sample was used to calculate the salivary flow rate (mL/min) and to determine the saliva pH using the Combur test (Roche Diagnostics Ltd., UK), according to the manufacturer's instructions. Oral yeast colonization was assessed microbiologically in all participants, regardless of the presence of clinical infection. An oral rinse technique described by Samaranayake and colleagues[11] was used for sample collections. Each subject was supplied with 10 mL sterile phosphate-buffered saline (0.1 M PBS, pH 7.2) in a universal container, requested to remove dentures if worn, and to swirl the 10 mL of PBS around the mouth for 60 seconds before expectorating the saliva-buffer mixture back into the container. The sample was sent immediately to the microbiology laboratory and inoculated onto a Sabouraud's dextrose agar (SDA) plate, and incubated aerobically at 37°C for 48 hours. The growth of any candidal colonies was recorded as a positive growth and the subject as a candidal carrier. The number of colonies on each plate was counted and the number of colony-forming units (CFU) per mL calculated, to indicate candidal density. The *Candida* strains in the isolated colonies were identified and tested for susceptibility to amphotericin B, fluconazole, nystatin, flucytosine, econazole, ketoconazole, and miconazole antifungals, using the commercial Candifast kit (International Micobio, France) according to the manufacturer's instructions. Basically, the Candifast test tray consisted of two rows with eight wells. The first row (the identification row) contained seven different sugars, the fermentation of which produced a color change in the phenol red indicator. The first well of this row contained phenol red, actidione, and glucose. Interpretation help was given by a colored chart included in the kit. The second row was the susceptibility row. The first well of this row was a growth control well and contained glucose. Wells two to eight contained glucose and each with contained an antifungal agent. The wells were inoculated with standardized inocula and covered with two drops of paraffin oil. The tray was incubated at 37°C. After 24 and 48 hours, the wells containing antifungal drugs (row 2) were examined, and the isolate was classified as 'resistant' (medium color: yellow and/or visible turbidity and/or a pellet), 'intermediate' (medium color: yellow-orange) or 'susceptible' (medium color:

red). The minimal inhibitory concentrations (MICs) corresponding to these three categories were not provided. Additionally, the identification of *Candida* species was confirmed by the conventional methods of germ tube production in horse serum,[12] following incubation at 35°C for two hours.

For diabetic patients, the blood sample was used to measure glycosylated hemoglobin concentrations (HbA_{1c}), which assessed the long-term glycemic control of that patient. For control subjects, the blood samples were used to measure the fasting plasma glucose level. Those with values of 7 mmol/mL or higher were excluded from the study, according to the WHO definition of diabetes.[13]

RESULTS

The diabetic and control groups were homogenous in terms of age, sex, dental status, and smoking habits (*P*>.05; Table 1). However, the control group showed a higher tooth brushing frequency than the diabetic group (*P*=.001). Also, there were no statistically significant differences between the two types of diabetic patients in any of the above-mentioned variables, nor in disease history, salivary flow rate or pH, except for the age, brushing frequency, and glycemic control (Tables 1 and 2). Type 2 diabetics were older than type 1 diabetics and had both a lower brushing frequency and glycosylated hemoglobin levels in comparison to type 1 diabetics [mean (SD) HbA_{1c}=8.95 (1.78) compared to 10.06 (2.06), respectively]. The diabetics had statistically, significantly lower salivary pH values compared to the controls (*P*=.015). However, there were statistically no significant differences in the levels of the salivary flow rates among the groups (Table 2, Figure 1).

The carriage rate or the frequency of detecting positive candidal growth was significantly higher in the diabetic patients than in the controls (*P*=.028; Table 2, Figure 2). The diabetic patients who were candidal

Table 1. Characteristics of the study population.

	Diabetics (n=150)	Controls (n=49)	*P**	Diabetics		*P**
				Type 1 (n=49)	Type 2 (n=101)	
Age (years)						
<30	12.7	14.0	.128	32.7	3.0	.001
30-50	37.3	52.0		30.6	40.6	
>50	50.0	34 .0		36.7	56.4	
Sex						
Male	41.3	38.0	.678	34.7	44.6	.250
Female	58.7	62.0		65.3	55.4	
Brushing frequency						
<1/day	16.0	4.1	.001	12.8	17.5	.673
Once/day	37.5	18.4		36.2	38.1	
>2/day	46.5	77.6		51.1	44.3	
Dental status						
Dentate	80.0	86.0	.346	84.8	77.8	.326
Denture wearer	20.0	14.0		15.2	22.2	
Smoker						
Yes	10.1	10.0	.98	12.8	8.9	.47
No	89.1	90 .0		87.2	91.1	

Values are percentages; *Chi-square test

Table 2. Candidal carriage, density, salivary flow rates, and pH among the groups.

	Diabetics	Controls	*P*	Diabetic patients		*P*
				Type 1	Type 2	
Candidal carriage						
Positive %	33.3	14.3	.028	51.2	25	.003
Negative %	66.7	85.7		48.8	75	
Candidal density (CFU/mL)						
Median (IQR)	0.0 (2000)	0.0 (0.0)	.200	1000 (3000)	0.0 (750)	.002
Salivary flow rates (mL/ mints)						
Median (IQR)	1.5 (1)	1.5 (1.13)	.479	1.0 (1.3)	1.5 (1)	.195
pH						
Median (IQR)	7 (2)	7 (1.0)	.015	7 (1)	7 (2)	.059

IQR: Interquartile range

Table 3. Distribution of candidal isolates in diabetics and healthy controls.

Candidal spp.	Diabetics	Controls	Diabetic patients	
			Type 1	Type 2
C albicans	31 (68.9)	2 (40.0)	14 (63.6)	17 (73.9)
C parapsilosis	3 (6.7)	0 (0.0)	1 (4.5)	2 (8.7)
C glabrata	5 (11.1)	1 (20.0)	3 (13.6)	2 (8.7)
C krusei	2 (4.4)	0 (0.0)	2 (9.1)	0 (0)
C tropicalis	1 (2.2)	0 (0.0)	1 (4.5)	0 (0)
Other yeast spp	3 (6.7)	2 (40.0)	1 (4.5)	2 (8.7)

Values are numbers and percentages

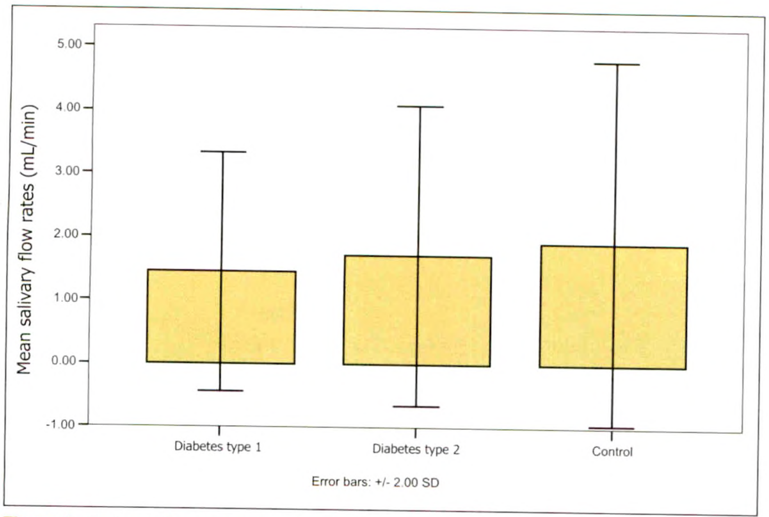

Figure 1. Salivary flow rates among the groups.

carriers harbored the yeast in higher densities than the control carriers, but the difference was not statistically significant (Figure 3). The mean candidal density for diabetic patients in this study was 3140.74 CFU/mL (SD=11 388.76). The number of patients with candidal carriage from the oral cavity was higher in patients with type 1 diabetes than in type 2 (*P*=.003). Similarly, the yeast density was higher in type 1 diabetics than in samples from type 2 diabetics, and the differences between the two groups were statistically significant (*P*=.002).

In both diabetic patients and healthy controls, the most frequently isolated yeast was *Candida albicans* (68.9% and 40%, respectively). The remaining isolates were found more frequently in DM patients as compared to the controls, including *C glabrata* (11.1%), *C parapsilosis* (6.7%), *C krusei* (4.4%), *C tropicalis* (2.2%), and other yeast species (6.7%) (Table 3). The in vitro antifungal susceptibility testing revealed that the yeast isolated from the diabetic patients had different rates of resistance to the seven tested antifungal drugs, except amophotericin B and nystatin, against which they had no resistance (Table 4). In contrast, in the healthy controls, none of the isolated yeast showed any resistance to the tested antifungal agents. When patients with different types of DM were compared, there was statistically no significant difference in the antifungal susceptibility (*P*>.05).

Statistical analysis showed no significant relationship between either the rate or density of candidal carriage and age, sex, brushing frequency, denture status, or smoking habits in any of the studied groups (*P*>.05). Similarly, medical and dental parameters such as disease duration, presence of diabetes complications, and salivary flow rates did not correlate significantly with the candidal carriage rate or density (P>.05). However, the rate of candidal carriage correlated significantly with the degree of glycemic control (as determined by the HbA_{1c} values) in type 2 diabetics and the salivary pH levels in healthy controls (Table 5). The candidal carriage rate correlated positively with the Hb_{A1c} values in type 2 diabetics, but negatively with the pH values in the healthy controls. In the type 2 diabetics, the candidal density correlated positively with the HbA_{1c} values (Pearson correlation=0.212; P=.047), and negatively with the salivary pH values (Pearson correlation=−0.338; P=.001).

DISCUSSION

A review of the literature published to date, on the relationship between DM and oral candidal infection reveals a continuing debate. Studies on candidal colonization are often contradictory, which may be a result of the variety of sampling techniques employed.[14-18] The present study comprehensively investigated microbiologically oral yeast colonization and evaluated the effect of some local and systemic factors, which could potentially influence the candidal carriage rate and density in diabetic patients. The reason for not conducting an oral examination for the clinical diagnosis of *Candida* was that it could be counted microbiologically without manifesting clinically and this was mentioned in the literature. A significant proportion of patients with no clinical evidence of oral candidiasis had >100 CFU/mL of yeast in their mouth rinses, suggesting that even an abundance of organisms in saliva might not initiate candidal infection.[5] The oral rinse sampling method was used as it was the most appropriate and sensitive technique for evaluating the overall yeast carriage compared to imprint culture, swab or saliva sampling.[11] The percentage of diabetic candidal carriers was nearly double that of healthy controls, confirming previous reports that candidal species were more prevalent in the oral cavity of diabetic patients than in healthy individuals.[4,14,17] In addition, the prevalence rates for candidal species in the present study, of 33.3% in diabetics compared with only 14.3% in the healthy controls, are similar to those in the previous studies, which reported a range of 18 to 80% for diabetic patients and 3 to 47% for healthy individuals.[5,14] The mean candidal density for the current diabetic isolates was 3140.74 CFU/mL, a value also within the range of candidal density reported among diabetic adult populations.[3,19]

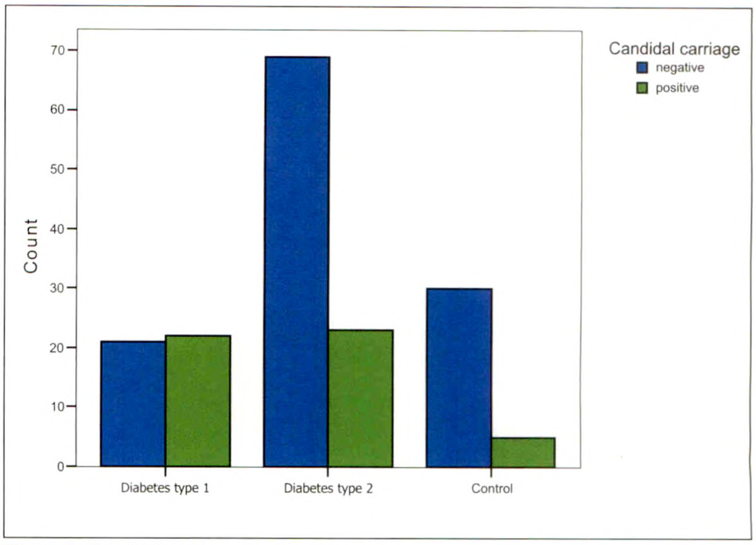

Figure 2. Candidal carriage among the groups.

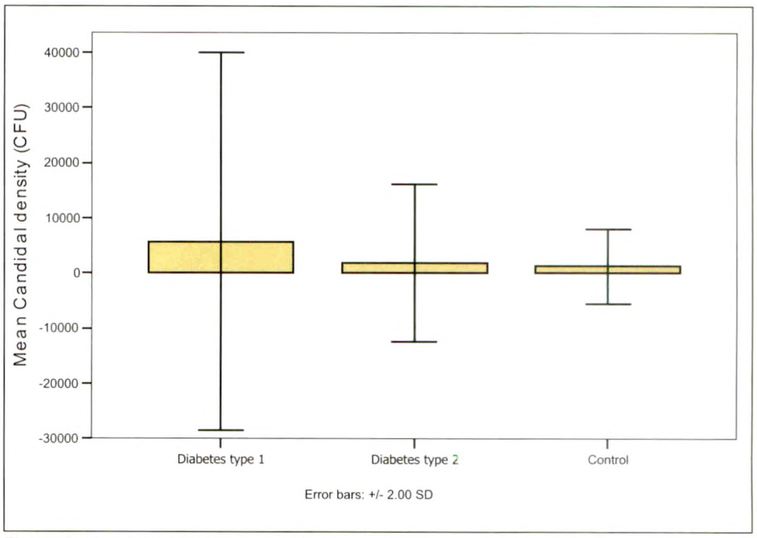

Figure 3. Candidal density among the groups.

Overall, our results herein agree with the other published results using the same sampling techniques. Diabetic patients have a higher prevalence of oral candidal carriage rate, but not density, compared to non-diabetics.[14,19,20] Although candidal density seems to be a useful predictor for the development of oral candidiasis in diabetic patients, previous studies have shown that candidal density, regardless of the methods used for sampling, does not correlate with the clinical evidence of oral candidiasis.[21] The higher carriage rate that we found in our study may be a better predictor for candidal infection in diabetics, but the question needs further investigation. The present study also agrees with the previously published reports that oral yeast

Table 4. In vitro susceptibility to antifungal agents.

Antifungal	Diabetics	Controls	Diabetic patients	
			Type 1	Type 2
Amphotericin B				
S	37 (100)	2 (100)	21 (100)	16 (100)
R	0 (0)	0 (0)	0 (0)	0 (0)
Nystatin				
S	37 (100)	2 (100)	21 (100)	16 (100)
R	0 (0)	0 (0)	0 (0)	0 (0)
Flucytosine				
S	31 (86.1)	2 (100)	17 (81)	14 (93.3)
R	5 (13.9)	0 (0)	4 (19)	1 (6.7)
Fluconazole				
S	25 (71.4)	2 (100)	14 (73.7)	11 (68.8)
R	10 (28.6)	0 (0)	5 (26.3)	5 (31.3)
Ketoconazole				
S	30 (85.7)	2 (100)	17 (85.)	13 (86.7)
R	5 (14.3)	0 (0)	3 (15)	2 (13.3)
Miconazole				
S	24 (68.6)	2 (100)	14 (73.7)	10 (62.5)
R	11 (31.4)	0 (0)	5 (26.3)	6 (37.5)
Econazole				
S	19 (73.1)	1 (100)	11 (73.3)	8 (72.7)
R	7 (26.9)	0 (0)	4 (26.3)	3 (27.3)

Values are numbers and percentages; S: sensitive, R: resistance

Table 5. Factors potentially influencing candidal growth in type 2 diabetics.

	Positive candidal growth		Negative candidal growth	
	Type 2 diabetics	Controls	Type 2 diabetics	Controls
HbA_{1c}	9.90 (1.88) *P=.006	NA	8.72 (1.65) *P=.006	NA
Salivary pH	6.8 (0.91) *P=.139	6.40 (0.55) *P=.003	7.10 (0.82) *P=.139	7.37 (0.63) *P=.003

Data are mean (SD); NA= not applicable, *ANOVA

colonization is higher in type 1 diabetics than in type 2,[4,15] although some investigators have failed to show a correlation between candidal carriage rates and density or the type of DM and the treatment used.[14,18,22]

C albicans was the most frequently isolated candidal species among the study groups, even as 31.1% of diabetic patients also carry other yeasts and candidal species. The latter include *C glabrata* (11.1%1), *C parapsilosis* (6.7%), *C krusei* (4.4%), and *C tropicalis* (2.2%). These findings are not surprising, as these organisms are the most common candidal species isolated from humans.[23] A similar diversity in candidal species has been reported previously among diabetic populations in Thailand,[24] Poland,[25] and Brazil.[26]

The most common antifungal drugs in the current clinical use, for treatment of oral candidiasis are polyenes (amphotericin B and nystatin) and azoles (miconazole, fluconazole, ketoconazole, and itraconazole), mainly used topically.[27] The therapeutic and prophylactic use of antifungal agents has given rise to alarming cases of antifungal resistance.[28] Interestingly, in vitro antifungal susceptibility tests revealed that none of the candidal isolates from the control group showed any resistance to the seven tested antifungals. However, oral yeasts isolated from diabetic patients displayed different resistance rates from the five azole antifungal agents, mainly miconazole and fluconazole. Such findings confirmed the results of other researchers reporting the emergence of fluconazole and, more generally, triazole resistance among different groups of immunocompromized patients in whom these agents were frequently used.[29] A recent in vitro study using the commercial Fungitest kit revealed no difference in susceptibility between candidal isolates from diabetics and non-diabetics to the six common antifungal agents tested.[27] However, they reported increased miconazole resistance among diabetic patients in London, which they justified as a consequence of the past widespread use of miconazole for the treatment of *Candida* induced denture stomatitis. In recent times, a number of rapid and easy-to-use commercial products have been introduced that identify yeast, and test antifungal susceptibility. The Candifast kit used in this study proved to be reliable for the identification of yeasts, but it had low reliability in antifungal susceptibility testing, which was attributed to high levels of subjectivity in the result interpretations.[30] However, the objective of this study was not to report accurate and clinically significant susceptibility, but to investigate whether there was an increase in in vitro antifungal resistance in the diabetic isolates. Therefore, this study should be extended to include more precise standardized tests with clear-cut objectives and interpretative criteria. Overall, based on the above findings we can say that the accurate identification of strains isolated from diabetic patients was especially important, because they were more likely to carry species other than *C albicans*, which might not be sensitive to certain antifungal agents. In addition the culture and sensitivity testing would add to the value of selecting the appropriate antifungal drugs, rather than prescribing any type of antifungal just for the presence of clinical manifestations of candidal infection.

The risk factors for oral candidal infection in diabetic patients are complex. Some authors emphasize the role of local factors such as low salivary flow rates[19] and pH,[15] smoking,[3,21] wearing dentures[15,22,31] and poor oral hygiene,[22] others focus on systemic factors such as patient age[22] or disease types,[4] degree of control,[15,31] and the presence of complications.[31] Overall, our results demonstrate that oral yeast carriage rate and density are not influenced significantly by patient age, gender, disease duration or complications. No statistically significant relationship was found between smoking habits, wearing dentures, brushing frequency, salivary flow rates and carriage rates or the density of candidal isolations. However, glycemic control, as determined by the level of glycosylated hemoglobin, positively correlated with the increased carriage rate and density of *Candida* isolates in type 2 diabetics. This may explain the higher carriage rates reported among type 1 diabetics compared to type 2 diabetics, because the former had higher mean HbA_{1c} values and poorer glycemic control. Hyperglycemia could contribute to the risk of oral *Candida* infection by increasing salivary glucose levels, which may promote overgrowth of candidal.[31] The results reveal that decreased salivary pH correlates with an increased candidal carriage rate in healthy controls, and increased candidal density in type 2 diabetics. Interestingly, although similar findings regarding salivary pH and glycemic control have been reported,[15] they have failed to show a significant difference in the candidal carriage rates or density between diabetics and non-diabetic controls.

In conclusion, diabetic patients had a higher oral candidal carriage rate, but not density, compared to non-diabetic controls. Although *C albicans* was the predominant isolate, a variety of other candidal species, with less susceptibility to azole antifungals, were identified in diabetics. Oral candidal colonization was significantly associated with diabetic type, glycemic control, and salivary pH, demonstrating a potential role of these factors in controlling candidal infections.

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original article

Quasispecies of genotype 4 of hepatitis C virus genomes in Saudi patients managed with interferon alfa and ribavirin therapy

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BACKGROUND AND OBJECTIVES: Many patients with hepatitis C virus (HCV) infection do not respond to antiviral treatment, possibly due to viral quasispecies. We aimed to investigate whether the quasispeices population could be used as a predictor of response to therapy in our patients.

METHODS: The quasispecies of HCV genotype 4 (HCV-4) were studied in 25 naïve Saudi patients at zero, three, and six months following interferon alfa and ribavirin combination therapy. Hypervariable region 1 within the E2/NS1 gene of the virus was analyzed by the single-strand conformation polymorphism (SSCP) technique after amplification.

RESULTS: Pretreatment DNA bands by SSCP (2-7 bands) were detected in all patients. In those who achieved a complete virological response within six months (viral load <0.2 Meq/mL; n=7), bands ranged from 2-6 (mean = 3.71±1.25). In six of these seven patients, the number of SSCP bands remained either the same or decreased sequentially. In those patients who did not respond (viral load >0.2 Meq/mL; n=18), the bands also ranged from 2-7; mean 3.77±1.73. In six of these non-responding patients, the SSCP bands remained the same or decreased sequentially. There was no significant difference between pretreatment quasispecies composition and response (*P*=.53). Two of the four patients with pretreatment high viral load and the same or decreased composition of quasispecies bands responded to the therapy.

CONCLUSION: Quasispecies in our studied patients cannot be used to predict responsiveness to treatment, but may offer an explanation for failure of most HCV-4 patients to respond to interferon alfa and ribavirin therapy.

Hepatitis C virus (HCV) belongs to the genus *Hepacivirus* of the family Flaviviridae. HCV constitutes a major health problem globally and it is estimated that 170 to 200 million individuals are chronically infected with HCV worldwide, with more than one million new cases every year.[1] HCV has a genome of nearly 9.6 kb that encodes a single large open-reading frame that yields a 3000-amino acid polyprotein. The large polypeptide is further post-translationally processed into several structural and non-structural proteins.[2,3] The HCV genome contains two conserved regions at its 5' and 3' ends that are essential for translation of the polypeptide and for viral replication.[4] HCV replicates at a very high rate resulting in a daily production of nearly 10^{12} virions, and the turnover of HCV virions is very short, reaching a maximum time of three hours.[5,6] During replication, mutations in the virus genome are generated due to the limited fidelity of the viral RNA-dependent RNA polymerase. As a result, HCV circulates in an infected individual as a heterogeneous population of different, but closely related genomes, known as quasispecies.[7,8]

The hypervariable region 1 (HVR1) of 27 amino acids, at the N terminus of the E2/NS1 region is considered the most variable in the HCV genome with a high frequency of mutations that exhibit significant amino acid variations between different genotypes and within the same genotype, during infection.[9] HVR1 is expressed on the surface of the virus and is thought to be the major epitope for neutralizing antibodies and for specific T cells.[10,11] It has been suggested that genetic variations in the quasispecies in HVR1 are the direct result of the selective pressure of the immune response during infection.[12,13] Many biological and clinical properties of the virus such as selection of the host immunity escape mutants and persistence of viral infection are attributed to the presence of quasispecies.[14-17] Furthermore, HCV quasispecies may influence the progression of liver diseases and the overall clinical and histological features after interferon alfa treatment.[18-20]

The current FDA-approved treatment of HCV infection is a combination of ribavirin and polyethylene glycol interferon (PEG-IFN), which includes two forms namely PEG-interferon alfa-2a (PEG-INTRON) and PEG-interferon alfa-2b (PEGASYS).[21,22] The attachment of PEG to interferon alfa extends its half-life and this reduces the number of injections of the drug. It was recently suggested that the quasispecies complexity of HCV may be a possible independent predictor of response to interferon alfa therapy. This conclusion was based on several studies that showed that the existence of a highly heterogeneous population of HVR1 sequences before treatment correlates with a lower rate of response to interferon alfa, independently of viral load and HCV genotype.[23,24] We investigated the effects of interferon alfa and ribavirin combination therapy on the evolution of HCV quasispecies in 25 of patients at the King Khalid University Hospital, with genotype 4 (HCV-4), who were enrolled in a previous study,[25] and determined whether the quasispecies population could be a predictor of sustained virological response in Saudi patients.

METHODS

Patient characteristics, clinical profiles, detection, quantification (viral load), and genotyping of serum HCV RNA were reported previously.[25] Twenty-five of these patients had left-over serum samples in our freezers that were used for this study. Each of the 25 naïve Saudi patients received ribavirin (Virazol, ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) at 1000 mg daily, in two divided doses and interferon alfa-2a (Intron-A, Schering-Plough International, Kenilworth, NJ, USA) at three million units, three times a week, for six months. All 25 patients had HCV-4 infection as confirmed by an InnoLiPA HCV II Genotyping Kit (Innogenetics, Belgium), which was used according to the manufacturer's instructions. HCV RNA was extracted from 100 µL of serum, using the Q1Aamp Viral RNA Kit (QIAGEN Inc. Santa Clarita, CA, USA) according to manufacturer's instructions. cDNA synthesis was performed as previously described.[26]

Primers for nested PCR were selected from E1 to E2 genes flanking the HVR1 region of the HCV genome.[27] The first round of amplification was carried out in a total volume of 50 µL using 20 µL of HCV cDNA preparation and 30 µL of PCR master mix (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM of each of the dNTPs (dATP, dGTP, dCTP, and dTTP), 40 pmoles of outer primers QP1 and QP2). The sequences of QP1 (sense) and QP2 (antisense) were: 5′-AGAATGGCCTGGGACATGATG-3′ (nt 1228-1248) and 5′-CAGTGTTTAAGCTGTCATTGC-3′ (nt 1565-1585), respectively. A 'hot start' PCR reaction was initiated by the addition of 1.25 U of Amplitaq DNA Polymerase. PCR cycling parameters consisted of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, for 35 cycles. The second round was performed with 40 pmoles for each of the inner primers, with 2 µl of the primary product as the template. The sequences of QP3 (sense) and QP4 (antisense) were: 5′-ACTGGGGTGTCCTCGTGGG-3′ (nt1334-1352) and 5′-AACAGCAATGGGAGCTGGCA-3′ (nt 1522-1541), respectively. All primer sequences were from the GenBank database Accession Number Y11604.1. Thirty cycles of amplification were performed using the same cycling conditions as the first round except that the final extension step was increased to 7 minutes, to allow the complete formation of duplex molecules. The amplified products (10 µL) were analyzed on 2% agarose gel electrophoresis, stained with ethidium bromide (1 µg/mL), and visualized under UV illumination.

The concentration of the PCR products was determined by using the DNA Dip Stick Kit (Invitrogen, San Diego, CA). SSCP was performed as previously described.[28] Briefly, 20 µl (15 µg) of the amplicons were mixed with equal volume of a solution containing 98% formamide, 0.05% bromophenol blue, and 2% glycerol, and denatured by heating at 94°C for 5 minutes, and immediately chilled on ice. The denatured samples were applied on 12.5% neutral polyacrylamide gels and separated at 200 V constant voltage at 25°C for 2 hours. The DNA bands were visualized by silver staining (PhastGel, Pharmacia).

The results were expressed as a mean ± SD. Differences in proportions were tested by the chi-square test. Mean quantitative values were compared by the t-test. All *P* values were two-tailed and a level of less than .05 were considered statistically significant.

RESULTS

E2/NS1 fragment containing a HVR1 of HCV-4 genome was amplified from the serum samples of Saudi patients at zero, three, and six months of interferon alfa and ribavirin combination therapy. The amplified products were used for SSCP analysis, to investigate the constitution of HVR1 quasispecies in the patients. A positive virological response (responsive patients or responders) was assessed by the disappearance of the viral serum RNA (viral load <0.2 Meq/mL) as long as six months of treatment. Figures 1a, 1b, and 1c illustrate the SSCP bands obtained.

The pretreatment number of the SSCP bands in patients who responded to the therapy ranged from 2 to 6 [mean number of SSCP bands=3.71±1.25], whereas, in the non-responding patients the range of SSCP bands was 2 to 7 [mean number of SSCP bands=3.77±1.73]. No significant difference was found between the pretreatment number of SSCP bands and response (*P*=.53). Seven patients (2, 4, 10, 13, 19, 20, and 25) were considered responders, six of them (4, 10, 13, 19, 20, and 25) were with a sustained virological response, as they had no detectable levels of HCV RNA at three and six months of treatment (Table 1). However, their SSCP bands varied. In all except one (patient 10), the SSCP band either remained the same or decreased. One of these patients (patient 25) had a low virus load (<1.0) before initiation of treatment, whereas, two patients (2 and 4) had a high virus load (>10.0). Three patients (13, 19, and 20) showed a decrease in the number of SSCP bands during the treatment period and had medium viral loads (1-10 Meq/mL) prior to initiation of treatment. Therefore, in patients who responded to interferon alfa and ribavirin therapy within six months, the HCV-4 quasispecies population in the majority of them either remained the same or decreased during the treatment period.

Eighteen of the 25 patients with viral load >0.2 Meq/mL were considered as non-responders (Table 2). Among them, four patients (16, 17, 23, and 24) showed the same SSCP band number during the

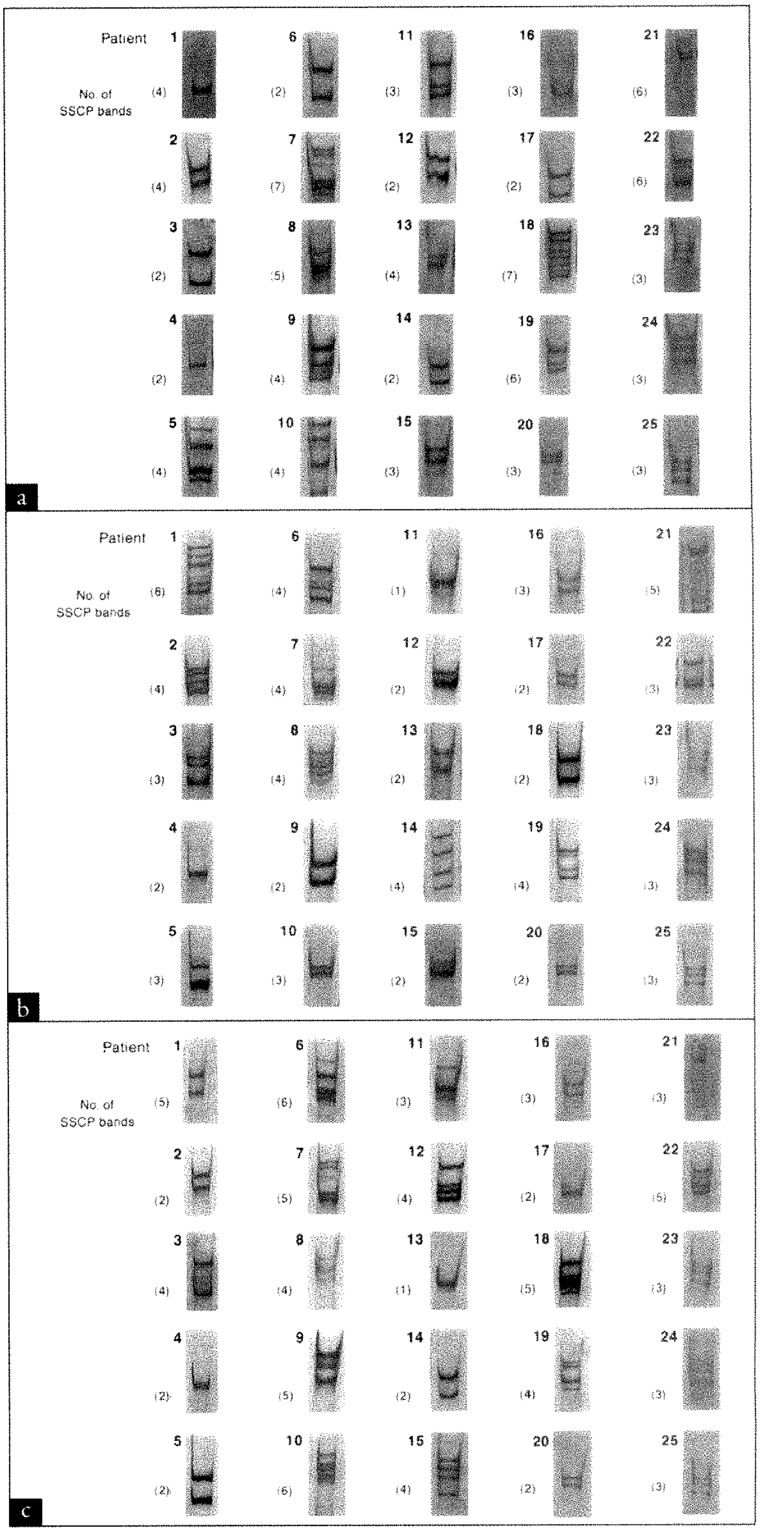

Figure 1. Quasispecies population in each of the 25-genotype 4, HCV-positive specimens. (a) pretreatment quasispecies population for all patients, (b) quasispecies population 3-months post-treatment, and (c) 6-months post treatment. Many bands may not be clearly seen as they were faint in the original gel due to lower concentration of the variants.

Table 1. Viral load and quasispecies population of responding patients.

Patient number	Genome copies/mL ×10^6 (number of SSCP bands)		
	Pretreatment	3 months	6 months
2	15.19 (4)	0.219 (4)	<0.2 (2)
4	12.91 (2)	<0.2 (2)	<0.2 (2)
10	4.83 (4)	<0.2 (3)	<0.2 (6)
13	3.84 (4)	<0.2 (2)	<0.2 (1)
19	1.63 (6)	<0.2 (4)	<0.2 (4)
20	1.63 (3)	<0.2 (2)	<0.2 (2)
25	0.49 (3)	<0.2 (3)	<0.2 (3)
Mean±SD of SSCP bands	3.71 (1.25)	2.86 (0.89)	2.86 (1.68)

Table 2. Viral load and quasispecies population of non-responding patients.

Patient number	Genome copies/mL ×10^6 (number of SSCP bands)		
	Pretreatment	3 months	6 months
1	40.76 (4)	4.22 (6)	14.38 (5)
3	13.07 (2)	3.20 (3)	3.50 (4)
5	9.77 (4)	<0.2 (3)	22.04 (2)
6	8.11 (2)	3.37 (4)	5.25 (6)
7	6.30 (7)	2.15 (4)	1.34 (5)
8	6.29 (5)	0.72 (4)	7.72 (4)
9	4.97 (4)	5.19 (2)	0.288 (5)
11	4.76 (3)	0.864 (1)	14.15 (3)
12	4.18 (2)	19.35 (2)	21.69 (4)
14	3.57 (2)	0.27 (4)	0.91 (2)
15	3.49 (3)	0.31 (2)	0.853 (4)
16	3.34 (3)	0.25 (3)	2.44 (3)
17	2.78 (2)	1.02 (2)	0.856 (2)
18	2.40 (7)	0.72 (2)	0.55 (5)
21	1.49 (6)	<0.2 (5)	28.96 (3)
22	1.30 (6)	3.47 (3)	1.08 (5)
23	0.56 (3)	<0.2 (3)	0.26 (3)
24	0.55 (3)	1.02 (3)	0.99 (3)
Mean±SD of SSCP bands	3.77 (1.73)	3.11 (1.23)	3.77 (1.22)

treatment period. Three patients (5, 8, and 21) showed a decrease and four patients (1, 3, 6, and 12) showed an increase in the number of SSCP bands during the treatment period. In seven patients (7, 9, 11, 14, 15, 18, and 22), there were a variable number of SSCP bands in which there was a decrease at three months followed by an increase at six months and vice-versa. Among the patients in whom the number of SSCP bands either remained the same or decreased, a medium viral load (1-10 Meq/mL) prior to initiation of therapy was noted. Two (6 and 12) of the four patients who showed an increase in the number of SSCP bands had a medium pretreatment viral load, whereas, the other two patients (1 and 3) had a high pretreatment viral load (>10 Meq/mL). All patients with variable number of SSCP bands (7, 9, 11, 14, 15, 18, and 22) had a medium pretreatment viral load (1-10 Meq/mL). Statistical analysis revealed no significant difference between pretreatment quasispecies composition and the response (P=.53).

DISCUSSION

Patients with HCV infection show many HCV genetic variants of the same genotype, known as quasispecies, due to the frequent mutation of the virus as it replicates. Persistence, resistance to therapy, and the transformation seen in these patients have long been attributed to these variants.[29] We did not study the details and outcomes of the treatment modalities, but investigated the importance of quasispecies constituents during the therapy of HCV-4 infection, to determine if it could be used to predict the treatment outcomes in our population. The treatment has been discussed elsewhere.[25] Quasispecies heterogeneity was shown in recent studies to be a possible independent predictor of response to interferon alfa therapy. Responsive patients typically possess less HCV quasispecies population in the pretreatment sera than do non-responsive patients.[30,31] A combination regimen of interferon alfa with ribavirin was demonstrated in clinical trials to produce higher rates of sustained virological, biochemical, and histological responses, particularly in patients with a relapse.[21,32] However, the role of HCV quasispecies complexity on the outcome of interferon alfa and ribavirin combination therapy for HCV-4 infected Saudi patients, has not been studied. In a previous study to assess the effect of combination therapy of interferon alfa and ribavirin on chronic HCV-4 patients, we reported a poor response among the patients, with 23% showing a sustained biochemical response and 12% showing a sustained virological response at the end of a treatment period of 24 weeks (6 months).[25] In the present study we utilized SSCP analysis, a rapid and sensitive method for determining HCV heterogeneity, to evaluate HCV quasispecies complexity in the sera of 25 patients infected with HCV-4, before initiation of combination therapy and to assess changes in the quasispecies population after three and six months of therapy. The SSCP analysis was carried out on amplicons derived from the HVR1 region. This region is known to be highly heterogeneous in the virus genome and is therefore considered to reflect most accurately the quasispecies nature of the virus in the serum of the individual patient.[13,33] Previous studies of HCV quasispecies, using SSCP and heteroduplex gel shift analysis, have identified two types of populations circulating in the serum of an infected individual.[34,35] The first type consists of simple quasispecies (single HCV species) characterized by a single SSCP band; the second is comprised of complex quasispecies characterized by multiple SSCP bands. Our SSCP data showed the presence of multiple bands in the pretreatment serum of both responder and non-responder patients. Hence the pretreatment serum of all patients examined contained variable quasispecies population, although statistically no difference was found in the average number of SSCP bands between responsive and non-responsive patients. The finding that none of our patients had homogenous HCV species in the pretreatment serum is significant and appears to be a unique feature of the HCV-4 virus not related to the pretreatment viral load. Indeed, despite the fact that patients in the study group were chosen randomly, a careful examination of the virus titer in the pretreatment serum supports this observation. Of seven patients who responded to the therapy, five had a low-to-medium viral load in the pretreatment serum. Of 18 patients who were non-responders, 16 also had a low-to-medium pretreatment viral load in the serum. Our findings were in agreement with previous studies carried out on patients with HCV-1b, who were also recognized to respond poorly to interferon alfa treatment.[36] Sakuma et al.[37] detected multiple SSCP bands in the pretreatment sera of seven of eight patients who did not respond to interferon alfa treatment. Gonzalez-Peralta et al.[38] also reported that among chronic hepatitis C patients treated with interferon alfa, HCV-1 (unspecified subtype) was associated with an increased number of quasispecies, resulting in poor response. Zekri et al.[39] in Egypt recently reported the effect of HCV-4 quasispecies on the treatment outcome. However, this study involved quasispecies from a less common area of the genome for this purpose (the 5' UTR) and not the usual HVR1, and the technique used was not the universally accepted SSCP. Another study reported that quasispecies were an important predictive factor for a sustained virological response for PEG-interferon alfa and ribavirin treatment.[40] However, the investigators worked on HCV-1 for 12 weeks and also reported that it was no better than a viral load as a predictive factor.

The presence of multiple HCV species in the pretreatment serum of HCV-4 patients could be one possible explanation for the resistance of HCV-4 virus to both the interferon alfa and interferon alfa and ribavirin combination regimen. The small number of subjects in our study does not allow for analysis of other possible factors such as side effects and histological grading, but the study did demonstrate that regardless of the presence of multiple HCV species in pretreatment serum, patients with stable viral populations or those with viral populations that decreased in complexity following treatment tend to respond to therapy. Nonetheless, the determination of quasispecies population cannot be used as a reliable predictor for effective therapy of HCV-4 infection.

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original article

Linkage and haplotype analysis for chemokine receptors clustered on chromosome 3p21.3 and transmitted in family pedigrees with asthma and atopy

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BACKGROUND AND OBJECTIVES: Genomic scan analyses have suggested that the chemokine receptor cluster (CCR2, CCR3, CCR5 <300 kb span) on the short arm of chromosome 3 may contribute to susceptibility to HIV-1 infection and to the expression of a number of inflammatory diseases. Two single nucleotide polymorphisms (SNP) and a deletion in these chemokine receptors have also been found in case-control studies to be associated with susceptibility for asthma and related phenotypes. We extended these case-control studies by establishing whether these polymorphisms were in linkage and linkage disequilibrium with asthma and related phenotypes using linkage and haplotype analyses.

METHODS: We genotyped 154 nuclear families identified through two child probands with physician-diagnosed asthma (453 unrelated individuals) including 303 unrelated parents and 150 unrelated children. Atopy was defined as a positive skin prick test (SPT 3 mm) to a panel of common inhaled allergens.

RESULTS: From a panel of ten known SNPs, only three polymorphisms: –G190A in CCR2, –T51C in CCR3, and a 32 bp deletion in CCR5 were found to occur at clinically relevant frequencies. All 154 families were used for haplotype analysis but only 12 nuclear families were eligible for linkage analysis. Both analyses confirmed that the mutations were in linkage with asthma, but not with atopy.

CONCLUSION: The chemokine receptor genes on 3p21.3 are significantly plausible candidate genes that can influence the expression of asthma. The previous association of the CCR5Δ32 deletion with protection from childhood asthma appears to be explained by linkage disequilibrium with the –G190A mutation in the CCR2 receptor gene.

Asthma is a complex disease which in childhood is frequently, but not invariably, associated with atopy,[1] and which is only associated with approximately half of all adult cases.[2] Although is likely that many candidate genes contribute to disease expression in view of asthma's high population prevalence, candidate polymorphisms are also likely to have high population frequencies and play relevant roles in biologically plausible mechanistic pathways. The chemokine pathway is among the many plausible pathways involved in the underlying inflammatory airway responses that are typical of asthma.

Chemokines, or chemotactic cytokines, are secretory proteins produced by leucocytes and tissue cells, either constitutively or after induction.[3] The two main subfamilies, CXC and CC chemokines (also termed α and β chemokines), are distinguished according to whether the position of the first two cysteines are separated by one amino acid (CXC) or are adjacent to each other (CC). Cysteines form two disulphide bonds (cys1→cys3 and cys2→cys4), which confer to the chemokines their characteristic three-dimensional folding.[3] Receptors for chemokines belong to the seven transmembrane-spanning receptor family and the majority are G protein-coupled receptors.

Chemokines have been implicated in both inflammatory and homeostatic leukocyte migrations.[4] In contrast, CC chemokines generally attract monocytes and lymphocytes but not neutrophils. Several CC members have been shown to induce eosinophil migration, including eotaxin-1, eotaxin-2, MCP-2, MCP-3, MCP-4, RANTES, MDC, and MIP-1α, and many of these same chemokines also act on basophils. The selectivity of the CC subfamily for T cells, monocytes, eosinophils, and basophils has led to numerous studies of their roles in allergic inflammation. Several members of the C, CC, and CXC chemokine subfamilies induce T-cell migration. Polarization of T-cells into TH1 and TH2 is characterized by differences in chemokine receptor expression. For example, subsets of TH2 cells have been shown to selectively express CCR3, which plays a role in basophil and eosinophil recruitment, reinforcing the suggestion that CCR3 is an important chemokine receptor in the expression of allergic disease. CCR1 and CCR5 have also been reported to be markers of TH1 cells and there is circumstantial evidence that TH1 and TH2 cells may recruit additional, polarized T cells by amplifying loops involving selective chemokine induction by IFN-γ and IL-4.[5]

It has been suggested that there may be some contribution of the functional mutations in the chemokine pathway in several diseases including rheumatoid arthritis[6], HIV-1 infection,[7,8] atopic dermatitis,[9] and childhood asthma.[10] Activation of different chemokine receptors in the same cell type may produce distinct signals, one example being CCR2 engagement by monocyte chemoattractant protein (MCP-1) and histamine release in basophils.[11] Particular interest has been directed to the role of receptor internalization in the infection of susceptible cells by HIV, a process that can be mediated by CCR5, CXCR4, or other chemokine receptors in a virus strain-dependent manner. Generation of chemokine receptor knockout animals has been instructive as mice deficient in MCP-1 or its receptor, CCR2, have impaired host defence, hematopoiesis, TH1/TH2 balance, monocyte-macrophage recruitment, and an increased formation of atherosclerotic lesions.[12] The CCR3 receptor is important in the movement of eosinophils, basophils, and possibly TH2 cells. Chemokine binding initiates rapid internalization but no signal transduction.

Most of the genes encoding CC chemokine receptors including CCR1 to CCR9 have been mapped to chromosome 3p21.3-p24, and of these, CCR2, 3, and 5 are clustered within a total distance of 350 kb on chromosome 3p21.3.[13] The coding region of the majority of chemokine receptor genes resides on one single exon, with the exception of CCR7, which contains two introns interrupting the coding region of the domain.

In view of the complex interdependencies, redundancies, and in particular, the close genomic distance between three of the strongest and most studied candidates in human disease expression, we assessed the linkage and the linkage disequilibrium between SNPs in CCR5, CCR2, and CCR3 in families at high risk for asthma and atopy..

We analyzed numbers of SNPs within the chemokine receptors CCR2, CCR3, and CCR5 using DNA pooling and allelic quantification (AQ) to identify two most common SNPs in our population. Three highly allelic single nucleotide polymorphisms were observed: –G/A substitution at position 190 of the CCR2 gene changes the amino acid at position 64 from isoleucine to valine of its protein, –T/C substitution at position 51 of the CCR3 gene results in a silent mutation at position 17 of its protein, and a 32 base pair deletion within the CCR5 gene clustered in chromosome 3p21.4 within a 300 kbp span were examined by the linkage disequilibrium and linkage analysis for asthma and atopy.

METHODS

Subjects

Population characteristics have been described in detail elsewhere.[14,15] Briefly, we identified 154 unrelated nuclear families (598 individuals including children and parents) containing at least two children and young adults aged 8-24 yrs, the majority of whom had a physician's diagnosis of asthma (PDA) and current symptoms (wheezing in the past 12 months). Lung function was measured by spirometry according to American Thoracic Society (ATS) standards (ATS; 1991). Bronchial hyperresponsiveness BHR was assessed with a methacholine challenge test and the concentration (mg/mL) was noted at which there was a fall in FEV_1 of 20% (PC_{20}).[16] All 154 families were white Caucasian and were drawn from a population for which the UK 2001 census showed that 84% were born in Scotland, 12% in other parts of the UK, and only 4% outside the UK.

Atopy was defined as the presence of at least one positive skin prick test (SPT) with a wheal size of ≥3 mm according to the European guidelines[17] for five inhalant allergens (cat, dog, house dust mite, grass, and *Alternaria*); referenced to a negative control. These five allergens were selected according to our own clinical survey and patient questionnaire for atopic patients. This survey confirms that these five allergens are the most common in our populations. DNA was isolated from EDTA-treated whole blood using the phenol-chloroform method.

Consent was obtained from each participant and approval was awarded for all the studies by the Grampian Research Ethics Committee, our local IRB.

DNA pooling and allelic quantification

This protocol has been described in detail elsewhere.[14,15] Briefly, each prePCR pool was amplified by PCR for all candidate SNPs for each gene in triplicate using the following PCR conditions: 50 μL containing 100 ng of genomic DNA; 10X PCR Buffer; 25 mM $MgCl_2$; 10 mM dNTPs; 10 pmol/μL of each primer (Table 1). For each run, 5 U of *Taq* DNA polymerase was used in 40 cycles of 95°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1.5 minutes.[14,15] The PCR products (postPCR pools) were inspected for clearly scored products using electrophoresis (1.5% agarose gels stained with ethidium bromide). Thirty microliters of each postPCR pool were then used to determine allelic frequencies using Pyrosequencing™ technology (PSQ).[18] The PCR products were prepared for PSQ using a PSQ96 Sample Prep tool and PSQ reactions were performed using the PSQt96 SNP reagent kit according to the manufacturer's instructions with two different sequencing primers for each SNP (Table 1); each run was performed in triplicate. Each PSQ96 plate contained one negative control (no template) and one positive control per genotype. The program, PSQt96 evaluation allelic quantification (AQ) software, was used to obtain the ratio of each separate allele peak height to the sum of the heights of both allele peaks. To allow the conversion of this peak height ratio to allele frequency for the DNA pools, a standard curve was established based on individual samples and the ratios from the pooled samples plotted against individual sample frequency. The equation of the linear regression best-fit line was then determined for each SNP and used to convert the allele peak height ratios to allele frequencies in the DNA pools. All eight known SNPs in genes encoding for CCR2 and CCR3 were assessed to identify those with a clinically significant population frequency in our population. CCR5 SNPs were not assessed as we had previously established the possible relevance of the CCR532 polymorphism in our population.[10]

Genotyping and sequencing

Those SNPs with possibly relevant allele frequencies (above 2%) (Table 2) were genotyped and sequenced in each individual. Primers and probes were designed by Assay-by-design (Applied Biosystem) and probe lengths were adjusted such that both probes had approximately the same melting temperature (67°C). The probe melting temperature was 7.0-8.0°C above the primer melting temperature of 60°C. Out of the two fluorogenic allele-specific probes used, one matched the wild-type sequence and other matched the mutant sequence. Each probe was labeled at the 5′ end with a fluorescent reporter dye and at the 3′ end with the quencher dye, NFQ. The reporter dyes used were VIC for the wild-type sequence and FAM for the mutant sequence. TaqMan universal master mix (Applied Biosystem) was used at 1X final concentration and in a volume of 25 uL along with 50 ng of genomic DNA and sequencing primers (Table 1). Optical plates (Applied Biosystem) were thermocycled in the Prism 7700 Sequence Detection System (SDS) for real-time detection and end-point analysis.

The CCR5Δ32 polymorphism was genotyped using a PCR assay as follows: PCR was performed in 25 μL containing 100 ng of genomic DNA; 10X PCR Buffer; 25 mM $MgCl_2$; 10 mM dNTPs; 10 pmol/μL of each primer (Table 1), and 5 U of Taq DNA polymerase. PCR conditions were 40 cycles of 95°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1.5 minutes. The PCR products, 232 bp for wild-type allele and 200 bp for the deletion Δ32 allele, were resolved by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

Statistical analyses

The LINKAGE package used to assess the likelihood of the recombination fraction between loci of interest expressed as the common logarithm of the ratio or lodscore which was calculated using the MSIM program. Multipoint linkage analysis was performed using the MLINK, LINKMAP, VITESSE, and LINKLODS computer packages.[19,20]

The most common application of linkage analysis is to identify the location of a gene responsible for a particular Mendelian inherited disease. We sought to estimate the linkage between three genetic loci in chromosome 3p21.3 and asthma.

Only 12 out of 154 families had heterozygous parents for all three polymorphisms within the CCR5,

Table 1. Candidate SNPs and DNA pooling and sequencing oligonucleotide primers.

Chemokines	Assay	Descriptions	5′-Sequence-3′
CCR2	SNP1,2,3	PCR-Forward primer	Biotin-CGGTGCTCCCTGTCATAAAT
		PCR-Reverse primer	AGCCCACAATGGGAGAGTAA
	SNP1	V64I-PSQ-Sequencing primer	TTTTGCAGTTTATTAAGATGA
	SNP2	V52V-PSQ-Sequencing primer	CCCACAAAACCAAAGATG
	SNP3	L45V-PSQ-Sequencing primer	AGTAGAGCGGAGGCA
	SNP 4	PCR-Forward primer	Biotin-GAGGCATAGGGCAGTGAGAG
		PCR-Reverse primer	CTGAACTTCTCCCCAACGAA
	SNP4	N260N-PSQ-Sequencing primer	TGTTCAGGAGAATGACAA
CCR3	SNP 1,2	PCR-Forward primer	Biotin-CACATGTGGCATCTTTGTTG
		PCR-Reverse primer	GGCCATCAGTGCTCTGGTAT
	SNP1	G21D-PSQ-Sequencing primer	CTTTTTCACAGAGCAGG
	SNP2	5′UTR-PSQ-Sequencing primer	TGAGGTTGTCATTTCACTT
	SNP 3	PCR-Forward primer	CACATGTGGCATCTTTGTTG
		PCR-Reverse primer	Biotin-GGCCATCAGTGCTCTGGTAT
	SNP3	Y17Y-PSQ-Sequencing primer	CCTTTGGTACCACATCCT
	SNP 4	PCR-Forward primer	CCTGCTCTGTGAAAAAGCTGA
		PCR-Reverse primer	Biotin-GATCATCACCACCACCACAT
	SNP4	T39C-PSQ-Sequencing primer	CCAGTTTGTGCCCC
CCR2	V64I	TaqMan-Forward primer	CGGTGCTCCCTGTCATAAATTTGA
		TaqMan-Reverse primer	GTCAGTCAAGCACTTCAGCTTTT
		TaqMan-Probe 1	VIC-ACATGCTGGTCATCCT-NFQ
		TaqMan-Probe 2	FAM-ACATGCTGGTCGTCCT-NFQ
CCR3	Y17Y	TaqMan-Forward primer	AGTTGAGACCTTTGGTACCACATC
		TaqMan-Reverse primer	ATCAGTGCTCTGGTATCAGCTTTT
		TaqMan-Probe 1	VIC-CCACGTCATCGTAGTAG-NFQ
		TaqMan-Probe 2	FAM-CCACGTCATCATAGTAG-NFQ
CCR5	Δ32	PCR-Forward primer	TGTTTGCGTCTCTCCCAG
		PCR-Reverse primer	CACAGCCCTGTGCCTCTT

Table 2. Candidate SNPs, position, function and observed allelic frequencies.

Chemokines	SNPs	Location	AA change	Function	Observed cases (%)	Observed controls (%)	NCBI (%)
CCR2	C/G	Coding Exon 3	V45L	Nonsynonymous	0.20	0.31	0.5
CCR2	G/T	Coding Exon 3	V52V	Synonymous	0.16	0.22	0.4
CCR2	A/G	Coding Exon 3	I64V	Nonsynonymous	6.3	21.0	15.2
CCR2	T/C	Coding Exon 3	N260N	Synonymous	0.7	0.63	1.41
CCR3	C/T	Coding Exon 4	Y17Y	Synonymous	14.2	3.5	16.9
CCR3	A/G	Coding Exon 4	D21G	Nonsynonymous	0.2	1.6	1.3
CCR3	T/C	Coding Exon 4	L39P	Nonsynonymous	0.1	0.5	0.4
CCR3	A/G	5'UTR	Unknown	Unknown	0.2	0.3	0.2

NCBI: National Centre of Biotechnology Information.

CCR2, and CCR3 genes, and were therefore eligible for linkage analyses.

Haplotype association

Haplotype analyses were constructed from population genotype data and using the Haplotyper program.[21] Linkage disequilibrium between each polymorphism was estimated by using the Fisher exact test and the 2BY2 program and the output generated by the EH (Estimate Haplotype) program.[22] Bonferroni adjustment on the number of independent comparisons[23] was made on the correlation coefficients for each of the haplotypes tested to correct for the number of comparisons made. The same approach was used to correct for the number of polymorphisms tested by using the D values instead of the correlation coefficients to estimate the relatedness of the polymorphisms.

RESULTS

Simulation of linkage was performed to check the power of this study to detect linkage with asthma status as the phenotype. Genotyping was simulated using the SLINK program[24,25] for polymorphic SNPs that were located at three-recombination distances (θ) from each other. The three values for theta were therefore 0.001, representing a 1% recombination rate; 0.05, representing a 5% recombination rate; and 0.50, representing a 50% recombination rate (no linkage between the any of the three hypothesized SNPs). The polymorphic SNPs had two-allele frequencies for each investigated gene and corresponded to heterozygosity of 35.3, 26.8, and 16.9% for the CCR5, CCR2, CCR3 polymorphisms respectively (data not shown). The hypothetical trait allele was assigned a frequency of 0.261, 0.195, and 0.135 for CCR5, CCR2, and CCR3 respectively. Expected and maximal lodscores at different values of recombination fraction as well as the probability that the maximal lodscore will exceed a given value were calculated from 200 replicates using the analysis program MSIM[24,25] (data not shown). The average lodscores are indicative of the information content of the pedigree studied for linkage analysis, whereas the probability that the maximal lodscore exceeds a given constant is a direct estimate of the power of the analysis. The simulation analysis showed that the pedigrees used in this study were capable of giving average and maximal lodscores of 1.57 and 6.08 respectively, with an estimated $\theta=0.05$ between all four proposed disease loci. The study also showed that there was 89.5%, 44.3%, and 10.0% probability of obtaining lodscores >1.0, >2.0, and >3.0 respectively, at true $\theta=0.01$, provided that the initial assumptions were correct. The probability of obtaining a false positive result, represented by the percentage of maximal lodscore values >3.0 when $\theta=0.50$ (no linkage), was found to be 0.0% in this study.

To determine the inheritance pattern at chromosome 3p21.3, we genotyped 453 individuals in multiplex families for three genetic markers. Two-point linkage analysis was performed using the program MLINK[26] which calculates two-point lodscores at different predefined recombination fractions (θ). The θ values were defined from 0.05 to 0.5 at an increment of 0.05. Multipoint linkage analysis was then performed to detect linkage to asthma using the LINKMAP, VITESSE, and LINKLODS computer packages (Table 3).[19,20] LINKMAP and VITESSE generate maximum log likelihoods as they move the proposed disease locus across a fixed gene map. Recombination fractions and gene order for the three genes used in this study were derived from the National Centre for Biotechnology Information (NCBI) database. Maximal log likelihoods were converted to multipoint lodscores using the program LINKLODS. The highest multipoint lodscore of 3.9 was obtained in pedigree 8 at CCR5Δ32 ($\theta=0.00$) and 0.22 at CCR3Y17Y ($\theta=0.00$) in all pedigrees.

Table 3. Two-point lodscore at $\theta=0.00$ from the CCR5Δ32, CCR2 V64I, and CCR3 Y17Y polymorphisms.

Pedigree	Two-point lodscore at θ=0.00		
	CCR5Δ32	**CCR2I64V**	**CCR3Y17Y**
1	1.26	–4.19	0.22
2	3.86	0.06	0.22
3	2.43	0.00	0.22
4	1.30	0.16	0.22
5	3.01	0.00	0.22
6	3.00	0.00	0.22
7	3.29	0.25	0.22
8	3.90	0.00	0.22
9	3.18	0.00	0.22
10	3.01	–0.39	0.22
11	3.89	0.00	0.22
12	3.74	–0.22	0.22

Haplotype associations

If the population contains only a limited number of disease-predisposing ancestral alleles, affected individuals should show an excess of those ancestral haplotypes. To search for such association, we examined each chromosome in multiplex families and determined the haplotypes for three genetic markers. Depending on the mode of action of the putative predisposing genes, the trait-associated chromosomes will also contain normal chromosomes but, nonetheless, should still be enriched for disease-predisposing alleles if chromosome 3p21.3 plays a role in that trait.

An EH algorithm computer program was used to enumerate all possible haplotypes of adjacent markers by considering all haplotype lengths. For each haplotype, we counted the frequency of its occurrence among trait-associated and controls. Visual inspection suggested a clustering of apparently identical segments between distinct haplotypes in the region of these chemokine receptors, possibly reminiscent of a conserved founder haplotype. Analysis was done of the relationship between the genotypes defined by the combination of CCR5Δ32, CCR2-G190A, and CCR3-T51C polymorphisms in relation to asthma with or without atopy. The frequencies of individuals and haplotypes meeting for unrelated parents and children, cases, and controls are shown in Table 4. The frequencies of haplotype analyses by EH algorithm for case-control parents and children displayed in Table 4 suggested a linkage disequilibrium between these polymorphisms ($\chi^2=14.62$, $P=.05$). The Fisher exact test statistical analysis was applied to calculate Haplotype association and asthma status. Cross-tabulation statistical analyses were applied to calculate haplotype association and asthma status. These analyses suggested that haplotype 2 was associated with a high risk of asthma ($P=.01$), and haplotypes 3 and 4 were associated with controls ($P=.0001$).

DISCUSSION

Genetic linkage analyses have been successful in the identification of genes involved in many single gene disorders such as cystic fibrosis and have also been used with limited success in disorders involving many genes and environmental factors such as hypertension[27], colon cancer[28], and diabetes mellitus.[29]

In the current study, an attempt was made to use genetic linkage analysis to assess the role of the chromosome 3p21.3 region in asthma with or without atopy. Computer simulation revealed the limited power of the pedigrees used in this study by showing that there was only 10% probability of obtaining a lodscore >3.0 for a marker located at $\theta=0.01$ from the hypothetical trait locus. Actual linkage analysis performed on the 12 pedigrees showed positive values for lodscores across the entire region of chromosome 3p21.3 with

Table 4. Haplotype analyses for parents and children.

Haplotype	SNPs			Parents (N=303)		Children (N=150)	
	Δ32	164V	Y17Y	Cases (%)	Controls (%)	Cases (%)	Controls (%)
1	1	1	1	58 (74)	164 (71)	61 (64)	38 (68)
2	1	1	2	6 (8)	9 (4)	14 (15)	4 (7)*
3	1	2	1	3 (4)	29 (13)**	5 (5)	5 (10)
4	1	2	2	0 (0)	2 (0.6)**	0 (0)	0 (0)
5	2	1	1	10 (13)	22 (10)	11 (12)	7 (13)
6	2	1	2	1 (1)	1 (0)	1 (1)	1 (2)
7	2	2	1	0 (0)	4 (2)	3 (3)	0 (0)
8	2	2	2	0 (0)	0 (0)	0 (0)	0 (0)
Total				**78**	**231**	**95**	**55**

*P<.01, **P<.001 (Chi-χ^2)

a maximum value of 3.90 at the CCR5 gene (θ=.00). High LOD scores for the Δ32 allele, compared to the theoretical maximum, might have been in part due to the layout of the parameter files and the fact that the Δ32 allele had a high allele frequency in the present population. Although a dominant effect was assumed in the analysis, other models, including recessive or partial penetrance, would have been unlikely to change the overall results because of the high allele frequency and the large number of affected individuals in the families studied. This might limit the detection of linkage, especially for a complex disease such as asthma.

Haplotype analyses with the EH program suggested significant linkage disequilibrium between hypothetical SNPs within chemokine receptors and asthma and supported the suggestion that the -G190A polymorphism of CCR2 was protective against asthma.[13,14] The relationship was analyzed between genotype defined by the combination of the CCR5Δ32, CCR2V64I, and CCR3Y17Y polymorphisms and asthma with or without atopy. The frequencies of individuals and haplotype meeting for unrelated parents and children cases and controls suggested linkage disequilibrium between these polymorphisms. The analysis also suggested that it was the CCR2-G190A polymorphism rather than the CCR5Δ32 that was associated with protection against asthma as this protective association was independent of its co-association with CCR532. The -T51C polymorphism in CCR3 was in significant linkage disequilibrium with the wild types of CCR5 and CCR2, but had an association with increased asthma risk, independent of its tendency to be in linkage disequilibrium with the above two wild type polymorphisms.

In summary, the present study demonstrated the utility of linkage analysis in asthma. However, the availability of large multigenerational pedigrees remains a major hurdle, especially in complex diseases such as asthma or atopy. Heterogeneity among different populations for genetic factors contributing to complex traits such as asthma or atopy also complicates linkage analysis.

The linkage and haplotype analyses using a panel of SNPs in three adjacent chemokine receptor genes as markers in this study suggested that there was an association between chromosome 3 and asthma. However, a larger data set would be required to confirm the suggestions from these haplotype analyses due to the low number of individuals in some of haplotype fields.

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A meta-analysis of highly active anti-retroviral therapy for treatment of plasmablastic lymphoma

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BACKGROUND AND OBJECTIVES: Plasmablastic lymphoma is a recently described B-cell derived lymphoma. The prognosis of plasmablastic lymphoma patients is usually poor. We performed a systematic review of the literature on the use of highly active anti-retroviral therapy (HAART) and the prognosis of plasmablastic lymphoma.

METHODS: A comprehensive search of relevant databases, including Medline, Embase, the Cochrane Controlled Trials Register, the Cochrane Library, and the Science Citation Index yielded ten randomized controlled trials. Trials were divided into two groups according to therapy. The rates of plasmablastic lymphoma were analyzed using a fixed-effects model. Sensitivity analyses (on publication type, statistical model) were performed to further detect and evaluate clinically significant heterogeneity. Tests of survival for plasmablastic lymphoma were also performed by using Kaplan-Meier method.

RESULTS: Meta-analysis result showed that the prognosis of plasmablastic lymphoma patients was statistically different in the patients receiving HAART in addition to chemotherapy and/or radiotherapy than in the patients receiving the chemotherapy and/or radiotherapy alone (pooled relative risk=3.04; *P*=.03). Survival analyses also displayed a statistically significant difference (χ^2=6.22, *P*=.013).

CONCLUSION: HAART in addition to chemotherapy and/or radiotherapy is effective in improving the prognosis of plasmablastic lymphoma. However, the small sample sizes increase the likelihood of bias in the studies in this meta-analysis, and therefore, the results should be taken cautiously.

Plasmablastic lymphoma (PBL) is a rare variant of a diffuse, large B-cell lymphoma, which typically presents in the oral cavity in immunodeficient patients.[1] More recently, the World Health Organization (WHO) has classified PBL, accepted as a new entity in the WHO classification of tumors of the hematolpoietic lymphoid tissues (4th edition), as an AIDS-related illness. According to the definition of WHO, PBL is a diffuse proliferation of large neoplastic cells, most of which similar to B immunoblasts, but in which all tumor cells have the immunophenotype of plasma cells (CD138 and VS38c positive).[2-4] PBL accounts for approximately 2.6% of all HIV-related non-Hodgkin lymphomas (NHL).[5] About 76% of PBLs are reported to be infected by EBV and 17% to 37% by Kaposisarcoma-associated herpesvirus (HHV8).[1,6-8]

The prognosis of PBL patients is usually poor, regardless of the site of origin, and the factors influencing prognosis are not clear. The clinical course is very aggressive with most of patients dying in the first year after diagnosis.[7,9] After a review of the literature, we concluded that highly active anti-retrovirus therapy (HAART) might be an important influencing factor on the prognosis of PBL. Therefore, we performed a meta-analysis to assess the effects of HAART on the prognosis of PBL.

METHODS

Only randomized controlled trials (RCTs) on the prognosis of PBL that included patients receiving treatment with HAART were eligible for inclusion in this study. All patients had to be diagnosed as having PBL confirmed by pathology, including some variants of PBL who were HIV negative. Patients with other variants of diffuse large B-cell lymphoma with plasmablastic features were not included in this study. Inclusion criteria for each study in the main analysis were: (1) a minimal clinical follow-up of 24 hours; (2) a minimal sample size of 2 per trial; (3) patients receiving chemotherapy and/or radiotherapy and/or HAART; (4) comparison patients not receiving HAART or patients receiving chemotherapy and/or radiotherapy alone.

We used the following sources for the identification of trials: the Cochrane Central Register of Controlled Trials, the Cochrane Library, PubMed, Embase, Science Citation Index and meeting abstracts, up to December 2008. Key words were 'plasmablastic lymphoma,' 'plasmablastic lymphomas,' 'highly active anti-retrovirous therapy,' 'HAART.' The search terms were MeSH terms and the search was limited to RCTs in English. Two reviewers (Guan and Zhou) independently checked the titles, abstract sections and keywords of every record retrieved. Full articles were retrieved for further assessment when the information suggested the studies were eligible. References in papers identified in the electronic search were manually searched for relevant trials not included in electronic databases. Each was imported into a bibliographic database EndNote (Version X1) and merged into one core database. The contents of that database were exported to the computer program Review Manager (Version 5.0 for Windows; the Cochrane Collaboration, Oxford, UK, *http://www.cc-ims.net/revman*).

Two reviewers extracted data on intervention and outcomes independently, using a pre-tested data extraction form that was adapted from a standard form provided by the review group. The data extraction form included general information, study characteristics, information on participants, interventions, baseline characteristics and measurements and outcomes. Differences in data extraction were resolved by consensus, referring back to the original article. If necessary, information was sought from the authors of the original studies. The included studies were divided into two groups according to whether they received chemotherapy or radiotherapy or HAART vs. non-therapy (subgroup 1), and to whether they received HAART in addition to chemotherapy and/or radiotherapy vs. receiving chemotherapy and/or radiotherapy alone (subgroup 2).

Assessment of each trail was performed by two investigators (Guan and Zhou) independently. Disagreement was resolved by discussion between the two reviewers with consensus or with consultation of a third reviewer (Zhang). In particular, the following quality criteria were assessed: randomization procedure, allocation concealment, method of blinding, handling of drop-outs (intention-to-treat analysis), quantity of dropouts, selective (skewed) dropout, and method of blinding outcome assessment (if applicable). Quality of the trials were assessed by the Jadad quality scale,[12,13] a composite scoring system that includes randomization, concealment, and patient withdrawal and dropout. Trials with a score ≥3 were considered of high quality.

Dichotomous data were expressed as odds ratios

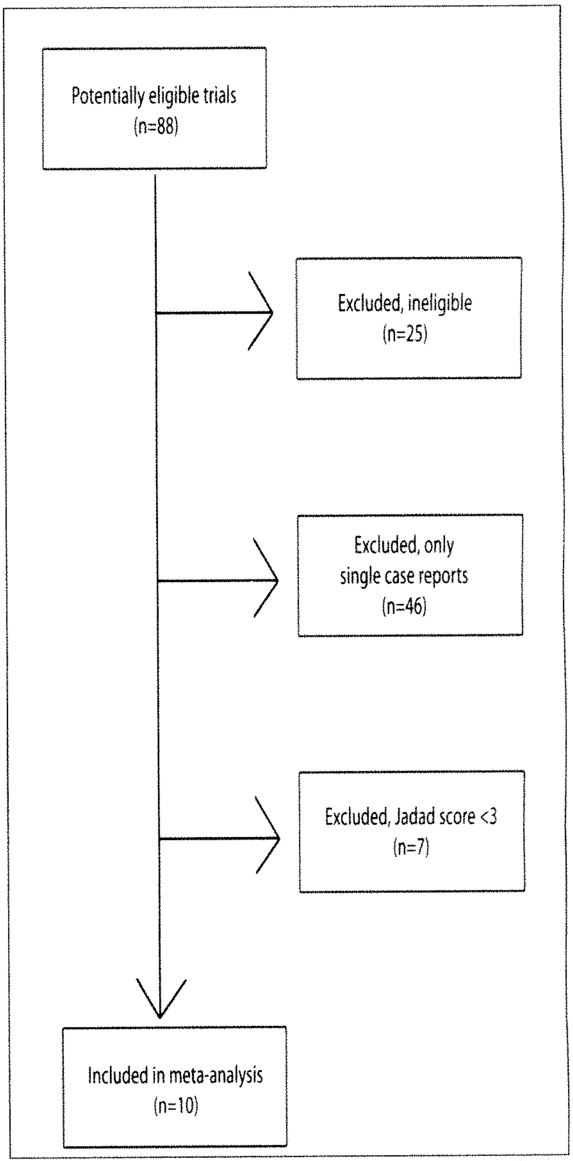

Potentially eligible trials
(n=88)
Excluded, ineligible
(n=25)
Excluded, only single case reports
(n=46)
Excluded, Jadad score <3
(n=7)
Included in meta-analysis
(n=10)

Figure 1. Study flow diagram of included and excluded studies.

(OR) or risk ratio (RR). Heterogeneity among the studies was examined using the DLQ statistic.[10] Pooled OR, RR and 95% confidence interval (CI) was calculated using the Mantel-Haenszel fixed-effects model when no statistically significant heterogeneity was detected. A random effects model was employed using the DerSimonian and Laird (DL) method if there was statistically significant heterogeneity. The chi-square statistic was set at $\alpha=0.1$, with $P<.1$ indicating a statistically significant difference. The Z score was set at $\alpha=0.05$, with $P<.05$ indicating a statistically significant difference.

Publication bias was estimated by examining the relationship between the treatment effects and the standard error of the estimate (S.E log RR) using a funnel plots. Quantification of the effect of heterogeneity would be assessed by means of *I* squared, ranging from 0% to 100% including its 95% confidence interval (CI) with significance set at $P>50\%$.[11] *I*-squared demonstrates the percentage of total variation across studies due to heterogeneity and was used to judge the consistency of evidence. The analyses were done with the Review Manager (Version 5.0 for Windows; the Cochrane Collaboration, Oxford, UK, *http://www.cc-ims.net/revman*). The statistically significant differences in each control group between subgroups were analyzed using the chi-square test, with $P<.05$ indicating statistically differences. Test of survival for HAART on the prognosis of PBL were also performed by using Kaplan-Meier method, with $P<.05$ indicating statistically significant differences. The date were processed by use of SPSS 16.0 (SPSS, Chicago, IL).

Several sensitivity analyses were performed to investigate the possible influence of the predefined quality criteria, including the statistical model (random-effects vs. fixed-effects). OR values were calculated for the sensitivity analyses. Several methods were employed to assess sensitivity. The effect of publication type was examined by excluding trials published in the form of abstracts only. The effect of study quality was examined by excluding trials with a Jadad score <3. The effect of sample size was examined by excluding trials with sample size <2.

RESULTS

A total of 88 studies were retrieved (Figure 1). Of these, 25 were irrelevant and excluded and 46 were excluded because they were only case reports. Seven studies were excluded because of poor data (Jadad score <3). Ten studies were included in our analysis (Table 1).[1,7,14-19] Subgroup 1 included eight trials. Subgroup 2 included two trials. A funnel plots suggested there was no publication bias (Figure 2).

Table 1. Characteristics of the 10 studies in the meta-analysis.

Study	Sample size	Research design	Jadad score
Subgroup 1			
Chetty 2003[14]	4	Unclear	3
Cioc 2004[15]	4	Unclear	3
Delecluse 1997[1]	16	RCT	4
Dong 2005[7]	14	RCT	3
Folk 2006[16]	5	RCT	3
Gaidano 2002[17]	12	RCT	4
Reidal 2008[18]	5	RCT	3
Teruya-Feldstein 2005[19]	12	RCT	4
Subgroup 2			
Gaidano 2002[17]	10	RCTs	4
Teruya-Feldstein 2005[19]	11	RCTs	4

Subgroup 1: Chemotherapy or radiotherapy or HAART vs. no therapy
Subgroup 2: HAART in addition to chemotherapy and/or radiotherapy vs. chemotherapy and/or radiotherapy alone.
Methodological quality: All studies were double-blinded; drop-out rates and randomization methods were clearly reported, but allocation concealment was unclear in every report.

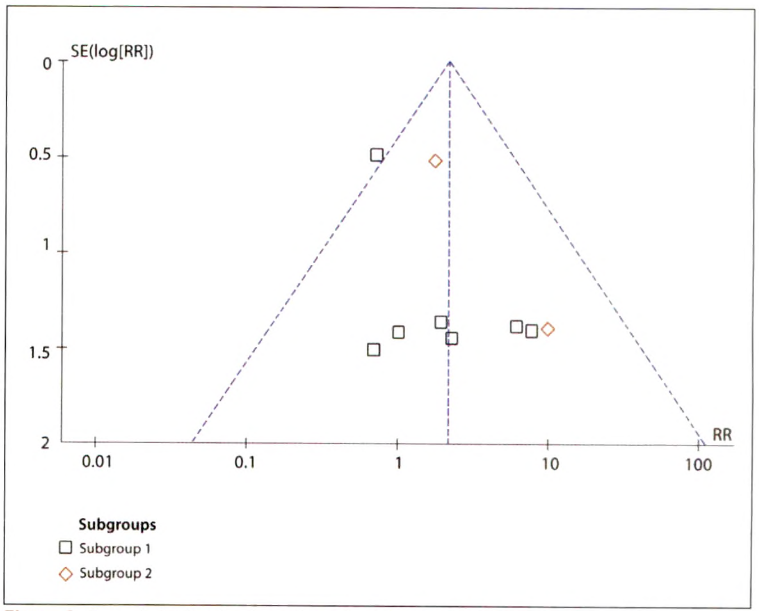


Figure 2. Funnel plot for the detection of publication bias for subgroups indicating no publication bias (broken lines are 95% confidence limits).

Study	Therapy		Non-therapy		Weight	Risk Ratio
	Events	Total	Events	Total		(95% CI)
Chetty 2003 (n=4)	1	1	0	3	5.1%	6.00 [0.40, 90.49]
Cloc 2004 (n=4)	1	1	0	3	5.1%	6.00 [0.40, 90.49]
Delecluse 1997 (n=16)	2	14	0	2	12.8%	1.00 [0.06, 15.99]
Dong 2005 (n=14)	1	12	0	2	12.4%	0.69 [0.04, 13.15]
Folk 2006 (n=5)	1	1	0	4	4.4%	7.50 [0.48, 117.16]
Gaidano 2002 (n=10)	3	10	0	2	12.0%	1.91 [0.13. 27.68]
Riedel 2008 (n=5)	1	3	0	2	8.8%	2.25 [0.13, 38.09]
Teruya-Feldstein 2004 (n=12)	6	11	1	1	39.4%	0.72 [0.28, 1.88]
Total (95% CI)		53		19	100.0%	1.87 [0.90, 3.87]
Total events	16		1			

Heterogeneity χ^2=6.85, df=7 (*P*=0.44); F=0%

Test for overall effect: Z=1.67 (*P*=0.09)

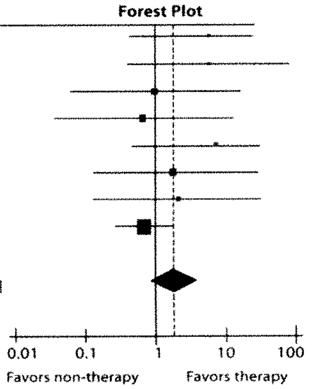
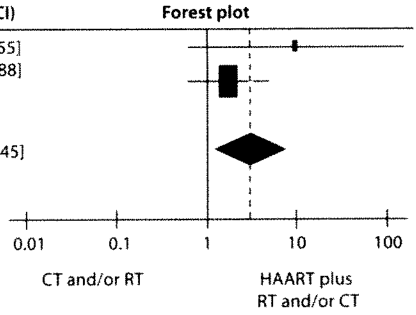


Figure 3. Forest plot for results in subgroup 1 studies, indicating no statistically significant differences between chemotherapy or radiotherapy or HAART vs non-therapy (size of data points represents sample size of study).

Study	HAART plus RT and/or CT		RT and/or CT alone		Weight	(95% CI)
	Events	Total	Events	Total		
Gaidano 2002 (n=10)[17]	3	4	0	6	16.0%	9.80 [0.64, 150.55]
Tervya-Feldstein 2004 (n=11)[19]	3	4	3	7	84.0%	1.75 [0.63, 4.88]
Total (95% CI)		8		13	100.0%	3.04 [1.09, 8.45]
Total events	6		3			

Heterogeneity χ^2=1.82, df=1, (*P*=0.18); F=45%

Test for overall effect: Z=2.13 (*P*=0.03)



HAART: highly active antiretroviral therapy, CT: chemotherapy, RT: radiotherapy

Figure 4. Forest plots for results of meta-analysis in subgroup 2 studies, indicating statistically significant differences for HAART in addition to chemotherapy and/or radiotherapy vs chemotherapy and/or radiotherapy alone (size of data points represents sample size of study).

Primary outcomes

Ten studies on PBL involving 93 patients were identified. No statistically significant heterogeneity existed in either subgroup 1 (*P*=.44) (Figure 3) or in subgroup 2 (*P*=.18) (Figure 4). In subgroup 1, the results showed no statistically significant differences in the patients receiving chemotherapy or radiotherapy or HAART than in the patients receiving non-therapy (30.2%, 16/53 vs. 5.3%, 1/19; pooled RR=1.87; 95% CI: 0.90-3.87; *P*=.09) on the prognosis of PBL. In subgroup 2, the results indicated statistically significant differences in patients receiving HAART in addition to chemotherapy and/or radiotherapy compared with patients receiving chemotherapy and/or radiotherapy alone (75%, 6/8 vs. 23.1%, 3/13; pooled RR=3.04; 95% CI: 1.09-8.45; *P*=.03) on the prognosis of PBL. No statistically significant differences were detected in the prognosis of PBL between the controls in either subgroup (5.26%, 1/19 vs. 23.08, 3/13%, χ^2=1.70, *P*=.19).

Sensitivity analyses

We selected a random-effects model for the analysis. Overall, the estimates of effect on prognosis of PBL were virtually identical for the sensitivity analyses and the meta-analysis (Table 2).

Survival analyses

Survival analyses were performed for the comparison of HAART in addition to chemotherapy and/or radiotherapy vs. the chemotherapy and/or radiotherapy alone, and for the comparison of HIV-infected vs.

Table 2. Sensitivity analysis for the possible influence of the predefined quality criteria.

Method	Subgroup	Patients	OR (95%CI)	Z	*P*	Heterogeneity		
						c^2	*P*	I^2(%)
Full text of publication	1	72	2.52 (0.78, 8.16)	1.54	.12	5	.73	0
	2	21	8.91 (1.16, 68.54)	2.1	.04	0.82	.37	0
Random-effect model	1	72	2.52 (0.68, 9.30)	1.38	.17	5	.66	0
	2	21	8.67 (1.03, 73.20)	1.98	.05	0.82	.37	0
Total events		**93**	**3.49 (1.27, 9.59)**	**2.43**	**.02**	**6.76**	**.66**	**0**

non-HIV infected patients. In the comparison of HAART, statistically significant differences on the prognosis of PBL were detected between the patients receiving HAART in addition to chemotherapy and/or radiotherapy and the patients receiving the chemotherapy and/or radiotherapy only (χ^2=6.22, degree of freedom=1, *P*=.013, log rank test with two-sided P values) (Figure 5). In the comparison of HIV vs non-HIV patients, no statistically significant differences was detected in the prognosis of PBL (χ^2=2.49, degree of freedom=1, *P*=.114, log rank test with two-sided *P* values).

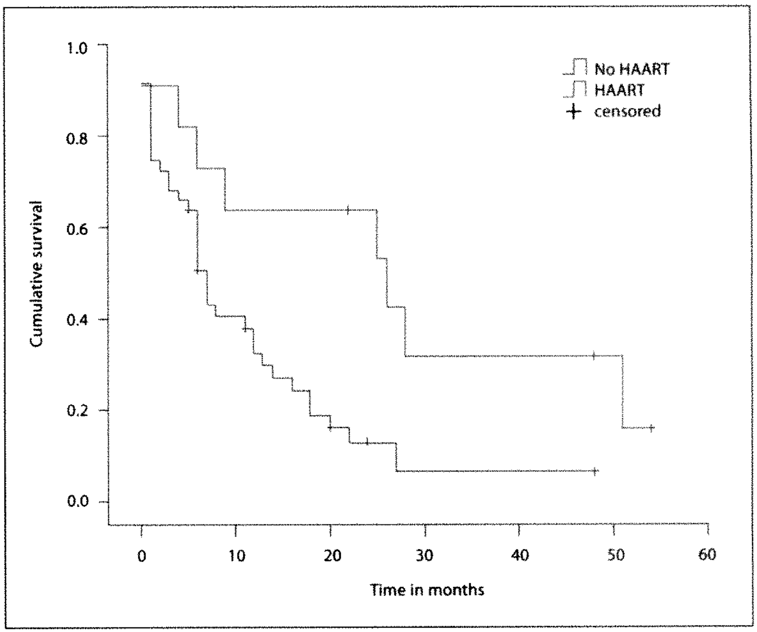


Figure 5. Kaplan-Meier survival analysis plots showing statistically significant differences between intervention groups and controls (χ^2=6.22, degree of freedom=1, *P*=.013, log rank test with two-sided *P* values).

DISCUSSION

The factors influencing the prognosis of PBL are not clear. Castillo et al[20] studied 112 published cases and found that EBV status, primary site of involvement, and clinical stage failed to show an association with survival using log-rank tests with two-sided P values. However, he did not perform a meta-analysis. On the other hand, there is no unified and effective agreement on the therapy for PBL.[18] Therapy depends on the clinical stage and the clinical symptoms caused by HIV infection. Moreover, there is no information on how choice of therapy relates to prognosis of PBL.[18,20-23] To clarify this question, we used the Cochrane systematic review method.

Our meta-analysis showed that HAART combined with chemotherapy and/or radiotherapy was more effective in improving the prognosis of PBL patients than chemotherapy and/or radiotherapy alone. However, The 95% CIs in some studies were very wide and the sample sizes small, which indicates a lack of precision in the estimate of relative risk. The small sizes of the trials might introduce bias in the results of the meta-analysis. There might also be a language bias, since only trials reported in English were included. The significant results in the survival analyses support the hypothesis that the addition of HAART to therapy has a favorable effect on prognosis, but the limitations associated with retrospectively accumulated data make it hard to draw firm conclusions. Therefore, the results should be interpreted cautiously. Larger, prospective studies are needed to further elucidate survival advantages for treatment that includes HAART.

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We are indebted to the authors of the primary studies included in this meta-analysis.

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Inter- and intraobserver variation between radiologists in the detection of abnormal parenchymal lung changes on high-resolution computed tomography

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BACKGROUND AND OBJECTIVES: Radiological and histological evaluations are affected by subjective interpretation. This study determined the level of inter- and intraobserver variation among radiologists for detection of abnormal parenchymal lung changes on high resolution computed tomography (HRCT).

METHODS: HRCT images of 65 patients known to have systemic lupus erythematosus (with clinical pulmonary involvement) were retrospectively reviewed by four nonthoracic radiologists (two with expertise in magnetic resonance [MR] and two general radiologists). Each radiologist read the scans twice, with an interval between readings of at least 6 months. The interobserver variation among the first and second readings of the four radiologists and the intraobserver variation of each radiologist's two readings were assessed by the kappa statistic.

RESULTS: There was good agreement between the first and second readings of each radiologist. There was moderate agreement between the two readings of one MR radiologist (kappa=0.482); the other three radiologists had kappa values that were good to excellent (0.716, 0.691, and 0.829). There was a clinically acceptable level of interobserver variability between all radiologists. The agreement was fair to moderate between the MR radiologist and the other observers (kappa range: 0.362-0.519) and moderate to good between the other three radiologists (0.508-0.730).

CONCLUSION: The interpretation of imaging findings of abnormal parenchymal lung changes on HRCT is reproducible and the agreement between general radiologists is clinically acceptable. There is reduced agreement when the radiologist is not involved on a regular basis with thoracic imaging. Difficult or indeterminate cases may benefit from review by a chest radiologist.

High-resolution computed tomography (HRCT) of the lungs is being increasingly used in the diagnosis and treatment of diffuse parenchymal lung disease (DPLD). Until recently, open lung biopsy was the most reliable guide to the likely outcome, with histological appearance suggesting inflammation indicating a relatively good prognosis and fibrotic change implying a poor outcome.[1-3] However, the invasive nature of open lung biopsy is a serious drawback. HRCT of the lungs is now being recognized as an excellent noninvasive technique for providing prognostic information and open lung biopsy is being relegated to situations where the findings on HCRT are equivocal. Noninvasive investigations such as pulmonary function tests, bronchoalveolar lavage, and chest radiography do not consistently identify reversible disease.[4]

The emergence of HRCT as a versatile diagnostic test is due to its diagnostic accuracy. Radiological and histological evaluations are affected by subjective interpretation and this observer variation can affect the reproducibility of a diagnostic test. With the increasing popularity and accessibility of HRCT, the majority of patients with interstitial lung disease are currently managed on the basis of HRCT observations, without histological evaluation. Thus, knowledge of observer variation in the interpretation of HRCT is needed.[5] We determined the degree of inter- and intraobserver variation in the detection of abnormal parenchymal lung changes on HRCT by quantifying the extent to which radiologists in a general hospital agree with each other with regard to HRCT findings.

METHODS

The HRCT images of 65 patients known to have systemic lupus erythematosus (SLE) and treated at the chest clinic in a general hospital were retrospectively reviewed by four radiologists. The four radiologists included two magnetic resonance radiologists (HK and OS) and two general radiologists (RA and PS). These participating radiologists, all working at a teaching hospital, had completed their general radiological training 8-16 years earlier at different institutions. The two MR radiologists had received special training in MRI for 1-2 years and had been mainly involved in MRI reporting for the past 4-6 years. All four radiologists were aware that the patients in the study had SLE, but no additional clinical information related to the respiratory system was made available to them when reviewing the HRCT images. Each radiologist read the scans twice, with an interval of at least 6 months between the two readings.

The radiologists determined whether the following radiological lung features of SLE were present: diffuse thickening, nodular thickening, alveolar honeycombing, perivascular nodularity, peribronchial nodularity, calcification, bronchiectasis, scarring, a ground-glass appearance, emphysematous bullae, prominent vasculature, pleural calcification, diffuse pleural thickening, nodular thickening with small and large nodules. After assessment, the results were recorded as normal lung parenchyma or abnormal lung parenchyma (if there were any parenchymal lung changes). The findings were described based on the location of the abnormalities (in the upper lobe, middle lobe (or lingular segments on the left), and lower lobe) and also according to the central or peripheral (outer third of the lung) nature of the abnormalities. The interobserver variation between the first and second readings of the four radiologists and the intraobserver variation between each radiologist's two readings were identified and the results assessed by the kappa statistic.

The images were obtained on a CT scanner (Somatom 4 Plus Spiral CT, Siemens, Germany), with 1-mm collimation at full inspiration. Scans were obtained at 10-mm intervals from the apices to the bases, with the patient in the supine position. The images were reconstructed with a high-spatial-frequency algorithm and photographed at window settings appropriate for viewing the lung parenchyma. All the images were evaluated on hard copy.

Interobserver and intraobserver variability in grading appearances on HRCT were quantified (Figures 1 and 2) using the kappa statistic, which measures agreement between observers while accounting for chance.[6] The data trends were evaluated by analysis of variance or the chi-square test. All statistical analyses were performed using STATA data analysis software (Computing Resource Center, Santa Monica, CA, USA). Observer agreement was categorized by kappa values as poor (<0.20), fair (0.20-0.39), moderate (0.40-0.59), good (0.60-0.79), or excellent (>0.80).[7]

RESULTS

The first and second readings (intraobserver variability) for each radiologist were compared (Table 1). There was statistically significant agreement between the first and second readings among all four radiologists. There was moderate agreement between the two readings of one of the MR radiologists (HK) with a kappa value of 0.482; the other three radiologists had kappa values of agreement that were good to excellent (0.716, 0.691, and 0.829). The *P* value of all four radiologists was .001 which indicates there is significant agreement between the observations of all the observers.

Agreement between radiologists (interobserver variability) was also compared for the two readings (Table 2). There was statistically significant interobserver agreement between all radiologists. This was fair to moderate between the MR radiologist (HK) and the other observers, with the kappa value ranging between 0.362 to 0.519. Among the other three radiologists the interobserver variability ranged from moderate to good (0.508 to 0.730).

DISCUSSION

Observer variability has been a problem ever since imaging began to contribute to the diagnosis of diffuse parenchymal lung disease, beginning as far back as the 1940s.[8,9] On chest radiography, the inability of the observer to compensate for under- and overexposure of films, varying classification systems, and a lack of familiarity with radiological manifestations were some of the problems faced. Further, previous studies assessing the role of chest radiography in fibrosing alveolitis have shown little correlation between appearances on chest radiography and histological findings.[10] These factors have resulted in an increasing reliance on computed tomography. With the widespread availability of HRCT scans for diagnosing diffuse lung disease, treatment is often initiated based on the interpretation of imaging findings, without recourse to histological confirmation. The aim of our study was to quantify the degree of intra- and interobserver variability between nonthoracic radiologists in the evaluation of HRCT images in patients with SLE attending a chest clinic in a general hospital. We focused on nonthoracic radiologists as we felt it would be more appropriate to assess observer variation among this group of radiologists who by and large report HRCT scans without access to dedicated chest radiologists and since they are increasingly providing the opinions on which decisions are made.

We used the kappa coefficient of agreement to evaluate observer variability as this accounts for chance agreement. The clinical significance of a kappa value depends upon its context and the values cannot be always compared between studies as it is dependent on disease prevalence.[11] In a recent review of studies that have used the kappa coefficient, the authors concluded that a value greater than 0.4 could be considered an acceptable level of observer variability.[7] We have followed the classification system used by Coblentz et al., where agreement is quantified as poor, fair, moderate, good, or excellent.[7] The second reading in our study could potentially be affected by the training effect and bias in the detection of findings as a consequence of the first reading. To minimize this, we ensured that there was a gap of at least 6 months between the two readings. The interobserver agreements on the two sets of readings showed similar trends and both sets were included in the analysis.

A few of the early studies performed to evaluate the diagnostic accuracy of HRCT also incorporated an evaluation of observer variation. However, these studies included many unusual diagnoses[12,13] and the number of observers were small.[13-15] Grenier et al.[12] and Lee et al.[14] reported fairly high kappa values of 0.78 and 0.75 for the diagnosis of diffuse interstitial lung disease. The kappa value is highly dependent on disease prevalence and thus studies may not be strictly comparable; nonetheless, these are higher values than were seen in our study and other studies in the literature. Collins et al.[10] assessed observer variation in diagnosing pattern type and disease extent in fibrosing alveolitis on HRCT scans and reported an interobserver variability on CT scan that was clinically acceptable. They demonstrated a higher level of confidence and less observer variability for CT scans than for chest radiography. Jokhoh et al[16] assessed the diagnostic accuracy of HRCT in idiopathic interstitial pneumonias and obtained a kappa value of 0.55. The differential diagnosis was however limited to just five types of idiopathic interstitial pneumonias, which probably increased

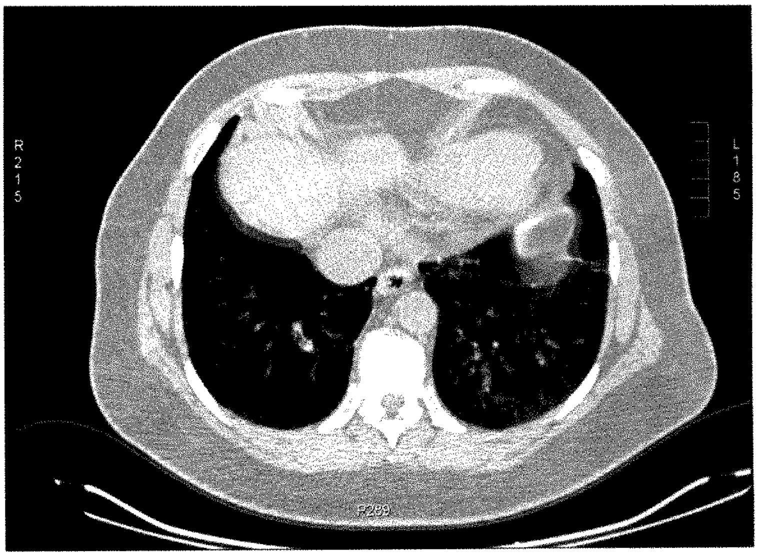

Figure 1. The image illustrating different observers' opinions evaluated as ground-glass appearance against improper breath-hold during scanning. Image used to evaluate observer variablity on ground-glass appearance vs. breath-holding during scanning.

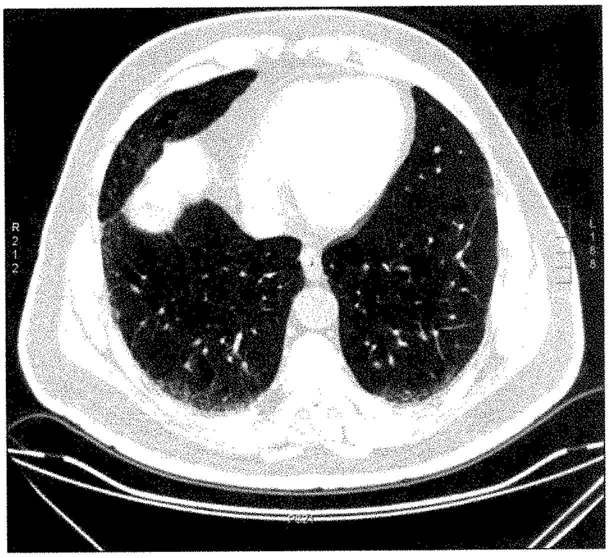

Figure 2. The image illustrating different observers' opinions as interstitial parenchymal change against dependent edema.

Table 1. Agreement between the two readings for each radiologist (intraobserver variability).

Radiologist	Agreement on normal	Agreement on abnormal	Disagreement between the two readings	Kappa value*	*P* value
HK	18/65	26/65	21/65	0.482	.001
OS	19/65	32/65	14/65	0.716	.001
PS	29/65	21/65	15/65	0.691	.001
RA	24/65	30/65	11/65	0.829	.001

*a value of 1 indicates perfect agreement

Table 2. Agreement between the radiologists (interobserver variability) for readings I and II.

Reading I	HK	OS	PS
OS	0.362		
PS	0.462	0.666	
RA	0.451	0.730	0.730
Reading II	**HK**	**OS**	**PS**
OS	0.551		
PS	0.339	0.508	
RA	0.519	0.620	0.604

the kappa value. In another study[17] of patients with suspected idiopathic pulmonary fibrosis (IPF) in which the need for lung biopsy was assessed, the agreement between radiologists regarding the presence or absence of IPF was 0.54 and 0.50, respectively. Thomeer et al.[18] evaluated the interobserver variation and accuracy of the diagnosis of IPF by respiratory physicians across six European countries who were given 179 HRCT scans for evaluation. They found the interobserver agreement to be fair to moderate between readers. The overall accuracy of the clinical diagnosis was good (87.2%). Our study is slightly different in that we specifically studied patients with SLE to evaluate the agreement between radiologists in the detection of findings. Radiopathological correlation was not performed as the objective was only to ascertain the degree of agreement in the evaluation of imaging. Our results are however similar with regard to the interobserver variation between radiologists in that the agreement between the observers (kappa value) ranged from fair to good. In our study, the intraobserver agreement ranged from moderate to excellent (0.482 to 0.829), indicating that the detection of relevant findings and interpretation of imaging appearances are reproducible. The interobserver agreement between one of the MR radiologists and the other three observers, was relatively poor, but it was still fair to moderate (the kappa value ranging from 0.362 to 0.519). This is no doubt due to the fact that this MR radiologist was not exposed on a daily basis to chest imaging. Nonetheless, the degree of interobserver variation is still comparable to that in other studies and shows that there is a reasonably good level of agreement between radiologists. The interobserver variability between the other three radiologists was even better, ranging from moderate to good (kappa: 0.508 to 0.730). We did not use a reference or gold standard in this study as the attempt was not to perform a radiological-pathological correlative analysis or to compare the radiological impression with a final proven diagnosis. Thus, a high level of agreement between observers does not necessarily indicate a high level accuracy of diagnosis.

The role, if any, of the better quality images produced by the newer and more versatile CT machines in the reduction of inter- and intraobserver variability has not been specifically studied, although this might be of some significance.

Significant variability between experienced histopathologists has been documented in the semiquantitative grading of interstitial fibrosis, intra-alveolar inflammation, and interstitial inflammation in open-lung biopsy samples.[19] The kappa values in all these instances were less than 0.30. In a study of diffuse lung disease by Nicholson et al.,[20] where pathologists could choose a diagnosis from a specified list of 15 categories, the kappa values for agreement between pathologists in tertiary referral cases was found to be 0.38. In comparison, the agreement among radiologists was found to be 0.34 for tertiary referral cases in the study by Aziz et al.[5]

Although we did not classify our patients based on the referral pattern from peripheral clinics, there would no doubt be a lower degree of agreement in tertiary referral cases. This highlights the fact that in difficult cases of DPLD it will not be sufficient to rely on either imaging or pathology in isolation. It is important in these situations to integrate the clinical information with HRCT and pathology findings (if available) before initiating treatment or formulating a final diagnosis.

In conclusion, our study quantifies the level of inter- and intraobserver variability in the detection of abnormal parenchymal lung changes of SLE. The interpretation of imaging findings is reproducible and comparable. The agreement between general radiologists is clinically acceptable. There is some variability in interpretation and reduced agreement between one MR radiologist (who was involved in thoracic CT imaging only infrequently) and the others. Difficult or indeterminate cases may benefit from review by a dedicated thoracic radiologist and, in some situations, a lung biopsy.

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Fetal weight normograms for singleton pregnancies in a Jordanian population

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BACKGROUND AND OBJECTIVES: Estimated intrauterine fetal weight (EIUFW) is important for reducing prenatal mortality and morbidity through early detection of faltering growth. Our objectives were to develop patterns of ultrasonically determined EIUFW by gestational age, for normal singleton pregnancies, and to assess the effect of a number of variables on EIUFW.

METHODS: Ultrasonically, EIUFW was obtained from 600 pregnant women who were at 20 to 42 weeks of gestation (WG). EIUFW was categorized into low weight and normal weight using the tenth and twentieth percentile as the cut-off points. Logistic regression was used to calculate the odds ratio and their 95% confidence limits for a number of risk factors hypothesized to be associated with low fetal weight. EIUFW percentiles (twenty-fifth, fiftieth, and seventy-fifth), by gestational age and sex, were calculated for singleton pregnancies.

RESULTS: Up to 32 WG there was no statistically significant difference between male and female fetuses in EIUFW. Between 32 and 39 WG males had significantly (P<.05) higher fetal weight than females. Charts of ultrasonically determined EIUFW by gestational age and sex for singleton pregnancies were created. A number of variables were significantly associated with EIUFW such as pregnancy weight gain, maternal hemoglobin level, frequency of antenatal visits, smoking status, and fetal sex.

CONCLUSION: Weight gain during pregnancy should be encouraged for pregnant mothers who gain less than one kilogram per month in the second and third trimester. A prospective study on a national representative sample in Jordan is needed to generate our own standards for fetal growth.

A simple and accurate method of estimating intrauterine fetal weight that can be easily applied to all pregnancies is an important means of reducing prenatal mortality and morbidity through early detection of faltering growth. Several investigators have shown that low estimated fetal weight is associated with high prenatal mortality and morbidity.[1] Birth weight is a composite of fetal growth and length of gestation, each of which has different contributions and different sequela. Removing the contribution of gestational age to birth weight is the first step in understanding the determinants of fetal growth.[2] Preterm birth is a prime predictor of neonatal complications, mortality, and developmental delay.[3] Birth weight may also predict both short- and long-term adverse outcomes. For example, higher birth weight among term infants is associated with birth complications[4] as well as a reduced risk of cardiovascular disease and hypertension in later life, but an increased risk of obesity.[5-8] In recent years, gains in neonatal survival have been most evident among low birth weight, preterm, and low birth weight (LBW) infants.[9] Most of the improvements in neonatal survival, since the 1980s, seem to be the consequence of decreasing birth weight-specific mortality rate, which occurred during a period of increasing preterm and LBW rates.[10]

The ultrasound has been used for the examination and evaluation of high-risk pregnancies and for the diagnosis of congenital malformations. During the last two decades ultrasound techniques have been improved and are implemented in most gynecology and obstetric clinics worldwide.[11] The biophysical profile was used to assess fetal well-being and to confirm fetal gestational age by measuring the biparietal diameter and crown rump length.[12] Other investigators have predicted intrauterine fetal weight using ultrasonographic measurement of the fetal abdominal circumference.[13] More recent reports have emphasized the usefulness of this measurement in monitoring normal fetal growth and in detecting intrauterine growth retardation.[14] All these studies were conducted in Western countries where perinatal medical care and ultrasonographic measurements are advanced.

The ultrasound has been recently introduced for evaluating pregnancies in most Jordanian antenatal clinics and hospitals, but in Jordan, reliable data pertaining to monitoring intrauterine fetal growth and development is lacking. Therefore, research in this area is needed to establish the fetal growth patterns in Jordan and to identify risk factors associated with the occurrence of low intrauterine fetal weight. Such research should be useful for establishing a national comprehensive program for early detection and management of low birth weight fetuses.

METHODS

This study was undertaken among pregnant women attending the antenatal clinic at Yarmouk University Health Center (YUHC) and Princess Badea Hospital (PBH). The sample in the two clinics was chosen consecutively. The formula used to estimate the sample size was $N=\mu\times$sample %, with N=sample size, μ=population size, sample %=0.05 (for population size more than 1000), or $N=12000\times0.05=600$.

A total of 856 pregnant women between 20 and 42 weeks of gestation, who attended the above two clinics for antenatal care or routine follow up were included in the study, and 256 pregnant women with anemia (hemoglobin level <9.5 g/dL), hypertension (diastolic pressure >90 mm Hg), vaginal bleeding after 20 weeks of gestation, multiple pregnancies and all presentations other than cephalic, were excluded. Our exclusion criteria depended on the obstetric and laboratory test results. Blood pressure was measured for each pregnant woman first and those who were suffering from hypertension (diastolic pressure >90 mm Hg) were excluded from the study.

Data were obtained from 600 pregnant women (300 from women attending YUHC and 300 from women attending PBH) during a visit to the clinic during the study period (June to December 2007). Information on maternal weight in the previous month and the date of last menstrual cycle was obtained from records (confirmed by an obstetrician), from a personal interview using a structured questionnaire that was first pretested and modified, and from ultrasound examinations by four obstetricians (two from each institution), who were not blinded. The ultrasonic measurements included fetal sex, fetal abdominal circumference (AC), and fetal biparietal diameter (BPD). Fetal AC and fetal BPD were used to estimate the intrauterine fetal weight. The BPD measurement was done for each fetus by obtaining a longitudinal section of the fetus by small sliding movements of the transducer on each side of the fetal spine. In this manner, a longitudinal section of the fetal head was obtained, which demonstrated a strong midline echo. By rotating the transducer through 90° a transverse section of the fetal head was obtained. If the section was not the required ovoid shape, the obstetrician made minor rotational adjustments until the shape was correct. If the midline echo was not at the exact center of the section, the obstetrician angled the transducer slightly. This corrected the angle of asynclytism. Slight movements of the transducer up or down the maternal abdomen would locate the correct section, which was then frozen. The BPD was measured by placing the horizontal component of the on-screen calipers on the outer aspects of the echoes from the fetal skull. This had to be at right angles to the midline and at the widest diameter.

Fetal AC is measured by scanning the fetus in a longitudinal plane until the fetal aorta is visualised in its course, through the fetal chest and abdomen. The transducer is then rotated through 90° at the level of the lower end of the fetal ribs. A minor adjustment is then made by moving the transducer up or down the fetal body until the section is obtained. The section is almost a perfect circle, containing only a short section of the umbilical vein in the anterior third of the fetal abdomen. The umbilical vein runs at an angle of approximately 45° through the fetal liver, so if there is more than a short length of vein visible in the section then the section is not at 90° to the spine and the fetal stomach is usually visible as a transonic area on the left side of the section. These parameters are used to predict fetal weight by using the fetal weight estimation table adopted from Shepard et al,[15] which was built according to this equation $BW=-1.7492+0.166\,(BPD)+0.046\,(AC)-0.002546\,(AC)\,(BPD)$.

Fetal weight was categorized into two categories: low weight and normal weight using the tenth and twentieth percentile as the cut-off points. Logistic regression was used to calculate the odds ratio and their 95% confidence limits, for a number of risk factors hypothesized to be associated with low fetal weight, after adjustment, for the effect of other variables. The data were examined for interaction by inclusion of interaction terms in the logistic regression models.

Fetal weight was also examined as a continuous variable. The independent effect of a number of variables on the fetal weight was calculated by using multiple linear regressions. The slope (the beta-coefficient) was the amount of change in the dependent (fetal weight) for one unit change in an independent variable, such as, pregnancy weight gain, pre-pregnancy maternal weight, and other variables, after adjusting the fetal gestational age. The Pearson correlation coefficient was used to identify the collinearity between independent variables, when two independent variables were highly correlated; one of these variables was excluded from the regression model. We performed all the analyses using the SPSS statistical package software, version 15 for Windows.

Table 1. Step-wise multiple linear regression analysis of the effect of several variables on the estimated fetal weight (each variable is adjusted for all other variables in the table.

Independent variables	Regression coefficients	*P*
Gestational age (weeks)	151.048	.001
Pre-pregnancy maternal weight (kg)	4.509	.001
Fetal sex (male vs. female)	54.146	.02
Pregnancy weight gain (high vs. low)	65.571	.001
Frequency of antenatal visits [less frequent users vs. frequent users (monthly)]	72.256	.001

Table 2. Logistic regression analysis of several variables on fetal weight below the 20th percentile versus fetal weight above the 20th percentile.

Variable	Odds ratio	95% CI	*P*
Entire sample (n=600)			
Maternal hemoglobin level (g/dL)			
-Less than 11	1.24	1.01-1.56	.05
-More than 11	1.00		
Frequency of antenatal visits			
-Frequent users (monthly)	1.69	1.20-2.38	.03
-Less frequent users	1.00		
>32 weeks gestation (n=268)			
Maternal hemoglobin level (g/dl)			
-Less than 11	1.98	1.03-3.82	.03
-More than 11	1.00		
Frequency of antenatal visits			
-Frequent users (monthly)	2.49	1.12-5.53	.02
-Less frequent users	1.00		
Smoking			
-Yes	3.42	1.09-10.7	.03
-No	1.00		

RESULTS

The comparison between the two samples obtained from PBH and YUHC showed a statistically significant difference between the two antenatal clinics. The differences between the two clinics were probably due to differeneces in the socioeconomic status. Of the 300 pregnant women who attended YUHC, 105 (35%) had a high monthly income (>800 Jordanian dinars, $1128 USD), 145 (48.3%) had a medium income (400-800 JD), and 50 (16.7%) had a low income (<400 JD), while just 25 (8.3%) of the 300 pregnant women who attended PBH had high monthly income, 127 (42.3%) had a medium income and 148 (49.3%) had a low income (P=.001). Most of the women who attended YUHC had a higher educational level than the women who attended PBH (P=.001).

Several variables were examined for the relationship with fetal weight after adjusting for fetal gestational age and other variables such as fetal sex, pre-pregnancy maternal weight, pregnancy weight gain, and frequency of antenatal visits, in a stepwise multiple linear regression analysis (Table 1). These relationships were also examined for fetal weight below the twentieth percentile using logistic regression, with adjustment for fetal gestational age (Table 2). Fetal sex was not related to the estimated fetal weight when it was included alone with gestational age in a linear regression model (P>.05). However, fetal sex was statistically associated with the estimated fetal weight (P<.05) in the presence of other variables, namely gestational age, pre-pregnancy maternal weight, pregnancy weight gain, and frequency of antenatal visits, where males had a higher fetal weight than females. In addition, weight gain during pregnancy was categorized into three levels according to Lin and Evans:[16] low weight gain (gain of 1 kg or less per month in the second or third trimester), medium weight gain (gain of 1 to 2 kg per month in the second or third trimester), and high weight gain (gain of more than 2 kg per month in the second or third trimester) showed a significant association with the estimated fetal weight (P<.001) after adjusting for gestational age, pre-

pregnancy maternal weight, fetal sex, and frequency of antenatal visits in the multiple linear regression model.

Frequent users of antenatal care services had a mean of 72.2 g less weight than less frequent users (*P*<.001). Frequent users were more likely to have low fetal weight fetuses compared to less frequent users (Table 2). For fetuses of greater than 32 weeks of gestation the results were similar. All the women had a hemoglobin level greater than 9.5 g/dL. Hemoglobin levels greater than 9.5 g/dL showed a statistically significant association with estimated fetal weight (*P*<.05). Women with hemoglobin levels <11 g/dL were more likely to have low weight fetuses compared to women who had hemoglobin levels more than 11 g/dL for the entire sample and for fetuses above 32 weeks gestation (Table 2).

Smoking status was not significantly associated with the estimated fetal weight when using bivariate linear regression analysis (*P*>.05). However, this variable was statistically significant for fetuses above 32 weeks of

Table 3. The fiftieth (median), twenty-fifth and seventy-fifth percentiles of fetal weight in grams for female and male fetuses in the entire sample (n=600).

Week of gestation	No. examined		Female			Male		
	Male	Female	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile
20	8	5	415.0	365.0	427.5	360.0	330.0	417.0
21	6	4	420.0	394.5	450.0	451.0	408.0	483.5
22	9	8	504.0	470.0	545.0	510.0	464.5	536.0
23	11	10	615.0	570.0	662.5	562.0	536.5	637.0
24	13	14	689.0	651.0	716.0	683.0	575.5	710.0
25	9	13	853.0	787.5	906.5	800.0	739.5	909.5
26	18	16	975.0	882.5	1050.0	935.0	902.5	980.0
27	15	19	1160.0	1050.0	1220.0	1240.0	1128.0	1310.0
28	17	18	1280.0	1170.0	1323.5	1335.0	1247.5	1386.0
29	14	15	1420.0	1352.5	1691.5	1488.0	1330.0	1645.0
30	11	20	1677.0	1644.0	1820.0	1770.0	1650.0	1818.5
31	18	15	1961.0	1810.0	2097.5	1895.0	1816.0	2103.5
32	16	19	2060.0	1995.5	2342.5	2100.0	1958.5	2282.0
33	20	17	2130.0	2013.5	2350.0	2206.0	2046.5	2335.0
34	18	15	2360.0	2210.0	2515.0	2380.0	2215.0	2677.0
35	13	17	2750.0	2450.0	2857.0	2770.0	2534.0	2879.5
36	15	13	2790.0	2510.0	2920.0	2920.0	2720.0	3030.0
37	17	15	3050.0	2912.5	3255.0	3060.0	2875.0	3160.0
38	18	13	3065.0	2940.0	3265.0	3200.0	2950.0	3287.5
39	19	21	3220.0	3070.0	3420.0	3265.0	3015.0	3490.0
40	9	10	3555.0	3491.0	3705.0	3270.0	3040.0	3505.0
41	2	2	4000.0	3495.0	4120.5	3500.0	3120.5	3702.5
42	2	3	4020.0	3510.0	4140.0	3611.0	3475.0	3850.0

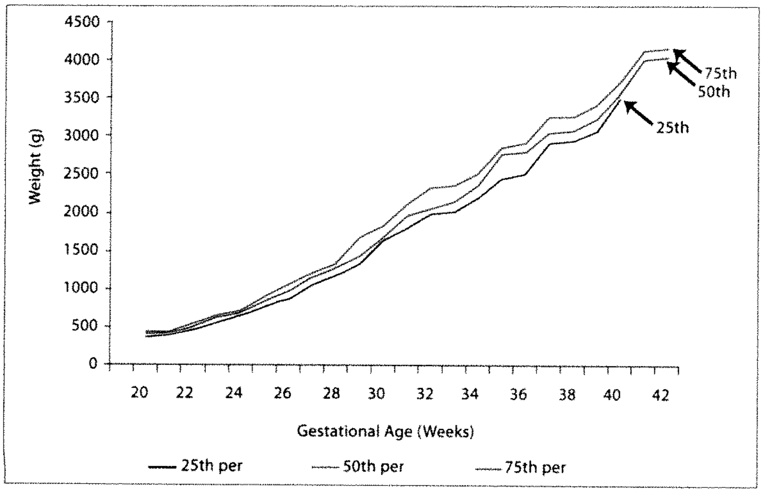

Figure 1. Estimated fetal weight percentiles for (female) singelton pregnancies.

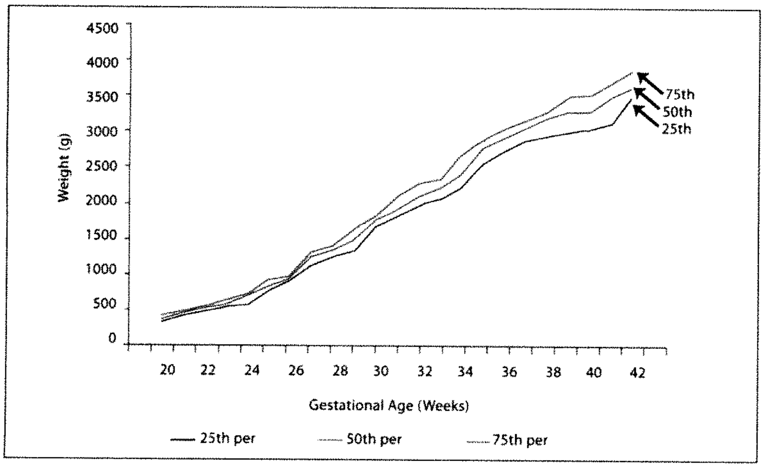

Figure 2. Estimated fetal weight percentiles for (male) singelton pregnancies.

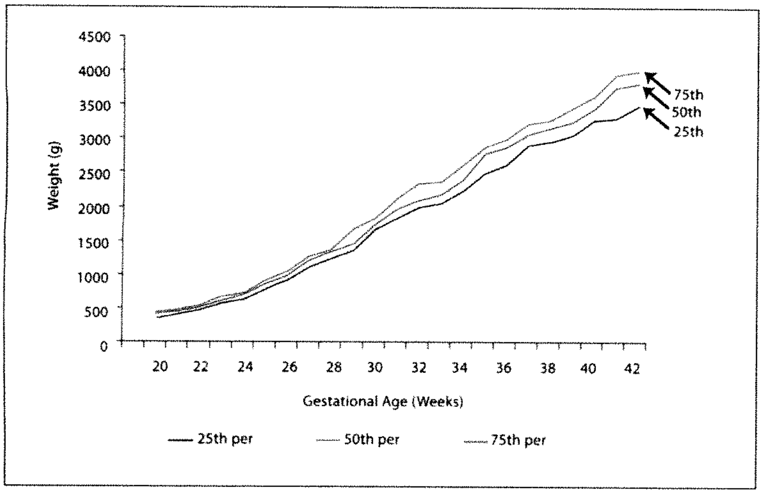

Figure 3. Estimated fetal weight percentiles for (male and female) singelton pregnancies.

gestation when comparing those below the twentieth percentile with the remainder (Table 2). Furthermore, pre-pregnancy maternal weight, maternal weight in the previous month of pregnancy, and maternal weight in the existing month of pregnancy were positively associated with the estimated fetal weight.

The same analyses were repeated using the tenth percentile of fetal weight as the cut-off point. None of the factors examined for a relationship with low fetal weight (below the tenth percentile) were statistically significant.

The fiftieth, twenty-fifth, and seventy-fifth percentiles of the estimated intrauterine fetal weights at 20 to 42 weeks of gestation are shown for female and male fetuses (Table 3). Up to 32 weeks of gestation there was no statistically significant difference between male and female fetuses. Between 32 and 39 weeks of gestation, males had a higher fetal weight than females ($P<.05$). After 39 weeks of gestation this difference was not statistically significant. The twenty-fifth, fiftieth, and seventy-fifth percentiles of the estimated intrauterine fetal weights throughout the pregnancy from 20 to 42 weeks of gestation are shown for females (Figure 1), males (Figure 2), and for both sexes (Figure 3).

DISCUSSION

Our results are consistent with the previous studies that show statistically significant relationships between fetal weight and variables such as maternal weight gain during pregnancy, pre-pregnancy maternal weight, and maternal weight in the previous month of pregnancy, and in the existing month of pregnancy, as well as maternal hemoglobin levels.[17] Copper et al.[18] reported that males had a higher fetal weight than females over the entire pregnancy. Others, however, found that this association was present only after 32 weeks of gestation.[19] In our study, males had a significantly higher fetal weight than females between 32 and 39 weeks of gestation, but after the thirty-ninth week of gestation this association was not significant. This could be due to technical difficulties in assessing the fetal weight after 39 weeks of gestation, when the liquor volume and visibility decreased leading to false positive results. In addition, there was more than one operator involved in the fetal measurements. Frequency of antenatal visits was inversely associated with estimated fetal weight, which was in contrast to the previous studies.[20] Frequent users of antenatal care were more likely to have low weight fetuses compared to less frequent users. One possible explanation for this unexpected result was that frequent users of antenatal care were more likely to have health problems, and therefore, felt a greater need to receive antenatal care than less frequent users. The results of this study showed a significant association between smoking and fetal weight, but only after 32 weeks of gestation, which agreed with the results of Rantakallo,[21] while no association was detected for 20 to 42 weeks of gestation. This finding was not consistent with the previous reports, which found that smoking during pregnancy resulted in low birth weight babies.[22] In the present study, only 5.5% of the mothers were smokers, and many were likely to be light smokers, which may explain the lack of association. Other investigators found a relationship between fetal weight and the intensity of smoking and changes in the pattern of smoking during pregnancy.[23] Unfortunately, no information was obtained in this study about these two aspects of smoking.

Estimated fetal weight percentiles by gestational age can be used to define the end points of epidemiological studies for factors affecting intrauterine growth or to assess the risk for infant mortality or morbidity. However, the figures presented in the study (Figures 1-3) are not intended to represent intrauterine growth, as the percentiles are based on cross-sectional measurements of estimated fetal weights.

By definition the tenth percentile has commonly been used as the upper limit for defining IUGR (intrauterine growth restriction) in fetuses, in epidemiological studies, but it need not be used to define IUGR for all applications. For epidemiological studies of fetal growth restriction, the fifth or even the tird percentile might be preferable. The percentile selected as most useful may differ between investigators depending on the purpose of the study, the population studied, the medical conditions being evaluated, or the set of intrauterine fetal weights for gestational age standards.[24] The third, fifth, and tenth percentiles have been used by clinicians to identify infants requiring more intensive observation. As an indicator of infants at the highest risk of poor perinatal outcome, the most appropriate percentile may change with gestational age.[25] Further study is needed, using linked intrauterine and live-birth infant files together with these new national norms, to determine which percentiles most accurately predict poor perinatal outcome.

Consistent with the previous studies such as that of Arbuckle et al,[26] our data revealed that the peak of the growth appeared between 30 and 40 weeks of gestation. Growth curves are potentially useful for several purposes in the clinical practice of obstetrics, gynecology, and pediatrics, as well as in public health and social sciences. Adequate fetal growth standards may be used as a basis for more informed clinical judgments and for a better interpretation of results of fetal monitoring. For example, in complicated pregnancies, when evaluating the best time for delivery, determination of the expected fetal weight at the present gestational age and the expected weight gain with each duration of delay may be helpful. Serial determinations of fetal growth by ultrasound and other methods could be used to assess growth restriction and the effects of different interventions. Finally, growth curves may be useful for selecting groups that might benefit from specific public health and educational programs and for evaluating the effects of such programs. The effects of genetic factors, illnesses, and personal habits such as smoking, diet, and addiction on fetal growth, can be evaluated, so that, potential parents can be counseled and effective public health programs can be initiated.

In conclusion, future mothers should be encouraged to have a normal weight before they become pregnant. Also, weight gain during pregnancy should be encouraged for pregnant mothers who gain less than one kilogram per month in the second and third trimesters. Nutrition education programs and food and vitamin supplementation should be provided for women with low hemoglobin. Cigarette smoking should be discouraged for all future mothers. Intrauterine fetal weight charts can be used to evaluate specific nutrition, medical care, educational programs, clinical judgments, and fetal monitoring, and to assess fetal growth restriction. These data should prove useful for researchers investigating the predictors and outcomes of altered fetal growth. A prospective (follow-up) study on a national representative sample in Jordan, or several follow-up studies in different governorates are needed to generate our own standards for fetal growth.

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review

The attitude towards disclosure of bad news to cancer patients in Saudi Arabia

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Disclosing the diagnosis or prognosis to cancer patients in Saudi Arabia can be a serious challenge to the physician in his daily clinic practice. The public attitude towards full disclosure is still conservative, and in order to appropriately deal with such an attitude, physicians need to deeply understand its sociocultural background. This article attempts to look into what governs the public attitude towards disclosure in Saudi Arabia as an example of what may affect attitudes in developing countries. It also brings some data from local surveys among physicians and patients as well as from public surveys to describe the changing trend in attitude over the years with a comparative analysis of the Western literature.

During the daily clinic practice, physicians occasionally find themselves confronted with situations of clear contradiction between what they have learned as the respectable ethics of medicine and what the public attitude dictates. In most of these situations, the family's request for non-disclosure of diagnosis and prognosis to the patient is the most contradicting and at the same time, the most difficult to manage. To appropriately respond to such a request, physicians need to deeply understand the sociocultural background of such an attitude.

This article attempts to look into what governs the public attitude regarding disclosure of full information to the patient in Saudi Arabia, as an example of the issue in many developing countries, and whether this attitude is amenable to the various psychosocial, educational influences or not.

Attitude is a hypothetical construct that represents an individual's degree of like or dislike for an item. Attitudes are generally positive, negative or neutral views of an "object": i.e. a person, behavior or event.[1] Most attitudes in individuals are a result of observational learning from their environment. The important question of whether attitudes change or not, was positively answered by many psychologists and socialists. Unlike personality, attitudes are expected to change as a function of experience. They can be changed through the tools of persuasion or by social influence like social proof and authority.[1]

The attitude in western societies in the past

The general attitude among physicians in the West in the recent past was not in favor of fully discussing the diagnosis or prognosis with patients. In 1953, a questionnaire administered to 442 Philadelphia physicians regarding the issue of disclosure of diagnosis found that only 31% of the physicians surveyed stated that they always tell their patients the details of diagnosis.[2] In 1960, a survey of 5000 physicians found that only 16% stated that they always tell the patients.[3] Another survey one year later of 219 physicians in Chicago found that 90% stated that they generally do not inform patients of their diagnosis.[4]

On the other hand, the public attitude was not so different. In 1948, a public survey for the American Cancer Society covered many aspects of public reaction to cancer. Information was collected by personal interviews of 1244 adult persons. To monitor for changes in public opinions, two repeat surveys were conducted in 1955 and 1962. One relevant question was "Supposing that a doctor finds out that a person has cancer, should the person be told?" Percentages of response with a clear yes were only 63%, 64% and 60% in the years of 1948, 1955 and 1962, respectively.[5]

There was a combined medical and largely sociocultural background for such a conservative attitude towards disclosure. This background constitutes many elements including:

The nature of the disease. Cancer was, and still is in many situations, viewed as a death sentence, and revealing the diagnosis to a patient was considered cruel and inhumane. Patients' relatives thought that disclosure would lead to loss of hope and that the patient would become devastated and crippled or even die earlier, if told about the diagnosis.

Paternalistic medicine and the principle of beneficence helped physicians to collude with the patient's relatives in not explaining the status and prognosis of the disease to the patient in many settings.

The extended family role in the face of a less patient autonomy, in the past and to some extent in the present time, in many non-Western countries, is a major component of the sociocultural background of such an attitude. Patients were viewed as extended family, and all decisions, including health-related decisions, are family-centered decisions.

The changing attitude: From 1960s and onwards

It is obvious that the attitudes of both physicians and the public have undergone major changes in the West in the past 50 years. In a questionnaire that was administered to a group of physicians who attended the 1999 Annual Meeting of the American Society of Clinical Oncology, participants were asked about difficulties they had when approaching stressful discussions and communication strategies used in giving unfavorable information.[6] The questionnaire was completed by 167 oncologists. Sixty-four per cent of them practiced in North America and Europe, and the remaining practiced in non-Western countries. In disclosing the cancer diagnosis and prognosis, physicians from Western countries were less likely to withhold unfavorable information from the patient at the family's request, avoid the discussion entirely, or use euphemisms. Another questionnaire given to both physicians and patients in the US and Japan confirmed this finding and revealed that both physicians and patients in the West are more inclined toward respecting patient autonomy and informed consent.[7] In that questionnaire sample, groups of Japanese physicians (n=400) and patients (n=65) as well as US physicians (n=120) and patients (n=60) were selected randomly. A majority of both US physicians and patients, but only a minority of Japanese physicians and patients, agreed that a patient should be informed of an incurable cancer diagnosis before their family is informed.

There is now a clear focus, both legally and ethically, on the issue of informed consent and patient autonomy. It is now an unshakable belief that telling the truth is a moral duty and that the patient has a need to know the truth to make decisions.

The change in the attitude in the west over the 1950s and 1960s was multifactorial in etiology. Revelation of the post World War II Nuremberg trials that disclosed experimentation on humans without consent, showed the need for the legal and ethical importance of informed consent.

The 1950s and 1960s was the era of social upheaval in the US, when movements for human rights were demanding rights for women, consumers, and finally patients, who began to demand to be fully informed about their diagnosis, prognosis, and treatment options. Furthermore, the increased optimism about the cure of some cancers due to advances in both surgical and radiation oncology and the beginning of medical oncology gave more treatment options and increased survival in many cancers. This in turn provided the momentum toward disclosing diagnoses to patients. On the physicians' side, there was greater recognition of communication as an effective means of enhancing patient understanding and compliance.

Saudi perspective

Attitudes are undergoing steady, albeit slow changes in many non-Western nations, including Saudi Arabia. Public education, in addition to the partial cultural openness due to the communication revolution, and worldwide globalization are having some effect in changing a few aspects of the sociocultural atmosphere in Saudi Arabia. Because of the complicated political, social and religious mix of values that govern the Saudi society, changes, if allowed to happen, are slow and follow a cautious path.

While the values of patient autonomy and informed consent are now rooted deep in the conscience of Western societies and control and shape the physician-patient relationship, these values are not prominent yet and have not become so influential in the Saudi society. The patient can frequently be thought of as an extended individual, with family members intimately involved with shared decision-making responsibility. Family members, and to a lesser extent friends, find themselves forced by their genuine cultural and mainly religious values to extend their help and support to their relatives or friends. They find themselves obliged, and frequently, allowed by the patient to do so by stepping forward and taking over some or all of the patient's responsibilities. This is the way they show sympathy and support to the sick relative or friend. They basically believe that patients (especially female patients) are very vulnerable and should not be left alone to handle the stress of knowing the bad news or the stress of making decisions. This supportive attitude, unfortunately, at the end may evolve into a dominating attitude that steals the patient's basic right of knowledge and decision-making. Nevertheless, many patients (especially old women) accept this situation, where the dominating relatives (sons, most of the time), become the major players. These patients trust their dominating relatives and literally hand over some or all responsibilities.

Bedikian *et al.* conducted a survey of 100 adult patients and companions referred to the Department of Oncology at King Faisal Specialist Hospital, Riyadh in 1984.[8] He found that only 16% of patients were told that they had "cancer" and 34% were told they had a "tumor". On the other hand, 69% of companions were told about the diagnosis of cancer. Another questionnaire was conducted almost 10 years later to assess the physician attitudes towards sharing information and decision-making with patients in the setting a serious illness.[9] Two hundred and forty-nine physicians from three different areas of the country participated in the study. Seventy-five per cent of physicians preferred to talk with close family members rather than patients.

In the face of such an attitude, oncologists from King Faisal Specialist Hospital, Riyadh,[10] tried to explain why physicians avoid telling their patients about their disease. They concluded that physicians may not know what to say or how to say it when they are about to break bad news. They may wish to avoid the difficulties of having to cope with a patient who is disturbed by the bad news, they may feel the patient simply will not cope, or it may take too much time and patience out of a busy work schedule.

To further monitor over the years for any evolving change in attitudes, a recent study was conducted to assess physician and public views in the country towards involving the patient versus the family in the process of diagnosis disclosure and decision-making. The study surveyed 321 physicians and 264 hospital attendees from six different regions.[11] In the case of a patient with incurable cancer, 67% of doctors and 51% of hospital attendees indicated that they would inform the patient in preference to the family of the diagnosis (P=.001). Assuming the family already knew, 56% of doctors and 49% of hospital attendees would tell the patient even if the family objected (difference not statistically significant). However, in the case of HIV infection, 59% of physicians and 81% of hospital attendees would inform the family about HIV status without the patient's consent (P=.001). The authors concluded that there is a need for greater recognition of patient autonomy among physicians.

These studies only assessed public and physician preferences and attitudes, which are clearly conservative regarding disclosure. There was at least one study that was conducted to assess patient preference specifically towards disclosure of diagnosis and prognosis.[12] A small survey of 114 patients in a teaching hospital in the Eastern Province revealed that almost all (113 patients) wished to know all information about their cancer and only one patient preferred to know partial information. All patients were against withholding information. Almost all patients wanted to know the benefits and adverse effects of therapy (98% and 99%, respectively), and all wanted to know about the prognosis of their disease. This study showed how the patient's preference is toward absolute disclosure while the public attitude is, in general, against full disclosure.

Current dilemma: Easy to say, difficult to do!

Every healthcare provider involved in the care of cancer patients knows how difficult it can be to disclose the diagnosis to the patient. Disclosure of prognosis, especially after failure of therapy, is even more difficult. To overcome such a difficulty, the following suggestions are provided:

- Disclosure has to be a systematic process and has to follow guidelines for breaking bad news. These guidelines are set up to make the process of disclosure smooth and fruitful. They should be taught in medical school and be a part of postgraduate training.
- Establishment of a support program for both patients and their families. There is no doubt that breaking bad news is a daily routine in oncology practice, putting a continuous psychological and emotional burden on physicians. Establishing a support program will definitely help physicians to cope with the situation better and enable them to perform the task in the optimal way.
- The argument for disclosure can be supported by many strong points, including the clear Islamic perspective to respect the patient and protect the patient's right to know and to freely make choices. It can also be supported by studies about patient preferences, which include evidence of benefit that is

now undebatable.[12] These benefits include building a trustful relationship with the physician, ability to make decisions or at least share in decision-making, improving compliance, and last but not the least, planning the end of life.

- More local research is needed to study all public, patient, and physician attitudes as well as signs of change and the underlying factors influencing change.

Finally, one of the most appropriate approaches that fit patients in many developing countries, including Saudi Arabia, is what James Hallenbeck and Robert Arnold described in an article titled "A Request for Nondisclosure: Don't Tell Mother".[13] This model of negotiation was used with the family and found to be very useful in the Saudi situation. This is a summary of the key points with minor modifications to fit the local clinic practice:

- Unlike in many parts in the world, physicians in Saudi Arabia, as per the local sociocultural background, frequently need to establish a physician-family rapport in addition to a physician-patient rapport.
- Respect the sociocultural background. Most of the time the family would like to know first. This can be acceptable provided that the patient is not denied the right to know.
- Try to understand the family's viewpoint and respond empathetically to their distress, keeping in mind that the aim is not to hide any information from the patient upon his or her request. Explain to them the benefits to the patient from disclosure and the practical difficulties associated with not telling the diagnosis.
- Explain the importance of truthfulness for you and negotiate how you will respond if the patient asks to be told the truth. Stress the point that, "if he or she asks me to tell the truth, I must do so".
- Talk to the family about what the patient would want and explain that "you are fine with a family member to be the decision-maker", if the patient concurs with the decision.

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Rate of conversion and complications of laparoscopic cholecystectomy in a tertiary care center in Saudi Arabia

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BACKGROUND AND OBJECTIVES: Problems during laparoscopic cholecystectomy include bile duct injury, conversion to open operation, and other postoperative complications. We retrospectively evaluated the causes for conversion and the rate of conversion from laparoscopic to open cholecystectomy and assessed the postoperative complications.

METHODS: Of 340 patients who presented with symptomatic gall bladder disease over a 2-year period, 290 (85%) patients were evaluated on an elective basis and scheduled for surgery, while the remaining 50 (14.7%) patients were admitted emergently with a diagnosis of acute cholecystitis.

RESULTS: The mean age of the patients was 41.9 (12.6) years. Conversion to laparotomy occurred in 17 patients (5%). The incidence of complications was 3.2%. The most common complication was postoperative transient pyrexia, which was seen in four patients (1.2%) followed by postoperative wound infection in three patients (0.9%), postoperative fluid collection and bile duct injury in two patients each (0.6%).

CONCLUSION: Laparoscopic cholecystectomy remains the 'gold standard' by which all other treatment modalities are judged. Conversion from laparoscopic to open cholecystectomy should be based on the sound clinical judgment of the surgeon and not be due to a lack of individual expertise.

Laparoscopic cholecystectomy (LC) is one of the most commonly performed laparoscopic procedures. It requires only a small wound (0.5-1 cm), causes relatively less pain, allows for early ambulation, requires shorter hospital stay and therefore allows early return to work, and is associated with an early return of intestinal movement and a lower incidence of incisional hernia.[1-3] The complications encountered during LC are numerous: some that are specific to this unique technique and some that are common to laparoscopic surgery in general. These include complications related to anesthesia; complications related to peritoneal access (e.g., vascular injuries, visceral injuries, and port-site hernia formation); complications related to pneumoperitoneum (e.g., cardiac complication, pulmonary complications, and gas embolism); and complications related to thermocoagulation. Specific complications of LC are hemorrhage, gall bladder perforation, bile leakage, bile duct injury, and perihepatic collection), and others such as external biliary fistula, wound sepsis, hematoma, foreign body inclusions, adhesions, metastatic port-site deposits, and cholelithoptysis.[4-6] We evaluated the causes for conversion and the rate of conversion from laparoscopic to open cholecystectomy and describe the diagnosis and management of the complications of laparoscopic cholecystectomy.

METHODS

We retrospectively reviewed 340 patients with symptomatic gall bladder disease who underwent LC at Khamis General Hospital between May 2006 and June 2008. Informed consent was obtained from all patients after the nature of the procedure and the possibility of the need for conversion from the laparoscopic approach to an open cholecystectomy was explained. A routine history was taken from all patients presenting for treatment of symptomatic gallbladder disease and all underwent a physical examination, laboratory testing, and ultrasonographic examination of the abdomen. Two hundred ninety (85%) patients were evaluated on an elective basis and scheduled for surgery, while the remaining 50 (14.7%) patients were admitted emergently with a diagnosis of acute cholecystitis. All acute cholecystitis patients (50) had right upper quadrant pain with localized tenderness. Twenty patients (40%) had fever of 38°C or greater on admission. An elevated white blood cell count (>10 000/mL) was present in 27 patients (54%). Ultrasonography showed wall thickening, pericholecystic fluid, and positive sonographic Murphy sign in 28 patients (56%). Patients admitted with a diagnosis of acute cholecystitis were treated with intravenous fluids, systemic antibiotics, and urgent surgery within 72 h of presentation. The clinical diagnosis of acute cholecystitis was confirmed by gross examination and histology in all 50 patients. All patients had surgery performed under general anesthesia. All were prepared and draped as for routine open cholecystectomy. A nasogastric (or orogastric) tube was inserted in those patients in whom there was a dilated stomach. An antibiotic (1 g Cephradine IV) was administered preoperatively, and a four-port technique was used in all the 340 patients.[7,8] Pneumoperitoneum, using carbon dioxide, was established using standard laparoscopic techniques through either a 'closed' or 'open' approach. Pneumoperitoneum was established by a closed technique in 210 patients (61.8%), while an open approach was used in 130 patients (38.2%).

Data are presented as the mean (and standard deviation) or percentage. Statistical analysis was performed using SPSS version 17 (Statistical Package for Social Sciences,Chicago, IL, USA). *T* test was used to compare continuous variables and nonparametric tests were used where applicable.

RESULTS

Of the 340 patients, 314 were women (92.4%) with a mean (SD) age of 41.4 (12.5) years and 26 were men (7.6%) with a mean age of 48.2 (13.19) years. The female-to-male ratio was 12:1. Indications for surgery were chronic calcular cholecystitis in the 290 patients (85.0%) who presented on an elective basis; the remaining 50 patients (14.7%) were admitted for treatment of acute cholecystitis. Ultrasonography demonstrated cholelithiasis in all the cases.

Conversion to laparotomy occured in 17 patients (5%) (Table 1). Perioperative complications leading to immediate or delayed laparotomy included injury to the common bile duct (two patients) and bile leakage due to a loose clip on the cystic duct in one patient. The incidence of complications was 3.2%. The most common complication was postoperative transient pyrexia, which was seen in four patients (1.2%) (Table 2).

Among the 17 patients who underwent conversion to laparotomy, one patient developed wound infection. Two patients who underwent LC developed port-site infection and were treated conservatively. The mean hospital stay was less in the LC group than in the 'converted to laparotomy' group (Table 3). Of the two cases of bile duct injury, one case was diagnosed intraoperatively and was converted to open laparotomy with repair of the duct and T-tube insertion until healing; the T-tube was removed 10 days later, with no morbidity. The other case was diagnosed postoperatively, the patient presenting with jaundice, abdominal pain, and distension. This patient made a good recovery after laparoscopic exploration, with intrabdominal drain insertion, clipping of the leaking duct opening, and referral to a higher center for hepaticojejunostomy. Twenty percent of the emergent laparoscopic cholecystectomies were converted to open cholecystectomy, while only 2.4% of elective LCs were converted to open laparotomy. The conversion rate was

Table 1. Indications for conversion from laparoscopic cholecystectomy to open cholecystectomy.

Factors contributing to conversion	Number of patients (%) (n = 340)
Contracted very small fibrotic adherent gall bladder	3 (0.9)
Gallbladder empyema	3 (0.9)
Unclear anatomy (acute cholecystitis)	6 (1. 7)
Difficult dissection	3 (0.9)
Gallbladder mass (cancer)	1 (0.3)
Common bile duct injury	1 (0.3)

Table 2. Postoperative complications.

Complication	Number of patients (%) (n=340)
Retained stones in common bile duct	2 (0.6)
Common bile duct injury	2 (0.6)
Biliary collection	1 (0.3)
Wound infection	3 (0.9)
Postoperative pyrexia	4 (1.2)

more in males (12/26, 46%) than in females (5/314, 1.6%) (Table 3).

Table 3. Characteristics of laparoscopic and open cholecystectomy groups.

	Laparoscopic (n=323)	Converted (n=17)*	*P*
Hospital stay	1.4 (0.2)	5.9 (3.0)	.003
Mean age	41.7 (12.5)	47.2 (14.8)	.001
Operative time	43.0 (9.7)	118.0 (26.3)	.001
Males (n=26)	14	12	.007
Females (n=314)	309	5	

Values are mean (standard deviation) or number of patients. *Ten cases were emergent (20% of emergency cases) and seven were elective (2.4% of elective cases).

DISCUSSION

Over the last two decades, LC has gained worldwide acceptance as the 'gold standard' in the surgical management of symptomatic cholecystolithiasis.[9] It is the commonest operation performed laparoscopically worldwide.[10] The conversion rate and complications associated with LC depend on the experience of the surgeon and the degree of difficulty faced during surgery. Different centers have reported widely varying rates of conversion to open operation (range: 1.5% to 6%).[11,12]

In our series, patient age was a significant predicting factor for difficulty during surgery and conversion to open cholecystectomy, with older patients being at greater risk for conversion to open cholecystectomy. Liu et al.,[13] Simopolous et al.,[14] and Kanaan et al.[15] reported that patients treated successfully by LC were generally younger than 50-60 years of age; in comparison, patients who required conversion had a mean age of more than 50 years and had a history of recurrent attacks of cholecystitis. Wiebke et al.[16] and Rosen et al.[17] reported that male sex was not a risk factor for difficulty during surgery or conversion. On the other hand, the findings of Kanaan et al.,[15] Simopolous et al.,[14] and Nachnani and Supe[18] are in agreement with our finding that male patients have an increased risk of difficult LC and that being a male increased the risk of unsuccessful LC.

As expected, an intraoperative finding of a chronically inflamed contracted gallbladder with a thickened wall during LC was associated with an increased conversion rate. In these cases, the dense adhesions signifed chronic inflammation and were certainly due to the repeated attacks of acute cholecystitis. Rosen et al.[17] reported that conversion from laparoscopic to open cholecystectomy was required in 5.3% of their patients. The main indication for conversion in their series was severe inflammation, preventing accurate delineation of ductal anatomy. Kuldip and Ashish[19] reported that the overall conversion rate was 0.4% of the total LCs performed and 1.7% of the difficult cases. They reported that dense adhesions in the region of the triangle of Calot was the leading cause for conversion to open surgery in 16.7% of their cases. Takegami et al.[20] reported a conversion rate of 13% in LC performed by general surgeons and 2% in LC performed by specialized surgeons, suggesting that the skill of the operator has a large influence on the conversion rate. Meshikhes et al.[21] and Al-Saigh et al.[22] from Saudi Arabia reported a conversion rate of 11% in their cases, the most common cause of conversion being difficult anatomy. Conversion from LC to open cholecystectomy was required in 5% of our patients. This compares favorably with the rates reported in the literature.

Since surgeons are reluctant to publish their own rate of complications, and since the complications of LC are treated in tertiary care centers, the precise magnitude of the problem remains uncertain. Serious complications of LC occur in fewer than 2% of all cases.[23] In our study the complication rate (3.24%) was within the range of published reports (0-8.6%), primarily because we included all complications, even the minor ones that were not related to the laparoscopic procedure itself. Placement of the Veress needle and the first trocar is usually linked to a complication rate that ranges from 0.3% to 0.5%.[24] A survey performed in the US showed a risk of vascular injury of 0.5%, while the rate of visceral injury was between 0% and 0.4%.[25] The reported incidence of bile duct injury is between 0% to 1% in LC.[22,23,26,27] In our study the incidence was 0.6%.

Wound infection, which usually involves the cannulation port and the epigastric port through which the gallbladder is extracted, occurs in 0.3% to 1%[27] of cases. In our study it occurred in three cases (0.9%): two in the port-site and one in the converted group. All were treated successfully with intravenous antibiotics. Postoperative fever occurred in 2.5% of patients in one study.[27] In our study it occurred in 1.8% of cases, and in all cases it was associated with either wound infection or immediate postoperative fluid collections. Other complications, such as those related to pneumoperitoneum or thermocoagulation, were not seen in our study. Port-site hernia was not found in our study, possibly because of the lack of long-term follow-up.

Conversion from LC to open cholecystectomy should be based on the sound clinical judgment of the operating surgeon and not be because of lack of expertise.

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Computer-aided endoscopic sinus surgery: a retrospective comparative study

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BACKGROUND AND OBJECTIVES: Endoscopic sinus surgery (ESS), markedly improved with the introduction of new preoperative imaging techniques, intraoperative visualization tools, and the use of surgical navigation systems. In this retrospective study we evaluated the usefulness of CT-guided endscopic sinus surgery and studied its advantages over conventional endscopic sinus surgery.

METHODS: We retrospectively reviewed the records of 60 randomly chosen patients with chronic rhinosinusitis (CRS) and moderate-to-severe sinonasal polyposis, undergoing endoscopic sinus surgery with surgical navigation (n=30) and without navigation (n=30). Data on the operative note, time of surgery, complications, and recurrence rate were collected and analyzed.

RESULTS: Of the 60 patients, 40 (66.7%) were diagnosed with CRS and 20 (33.3%) had allergic fungal sinusitis. Primary surgery was performed in 37 (61.7%) and revision surgery was performed in 23 (38.3%) cases. The computer-aided surgery (CAS) group included 28 (93.3%) patients with extensive disease and 12 (40%) with bone erosions, with intraorbital or extradural extension, while the non-CAS group included 24 (80%) patients with extensive disease and seven (23.3%) with bone erosions, with intraorbital or extradural extension. The average operative time was approximately 13 minutes greater in the navigation group, with significant improvement in the recurrence rate (n=11, 36.7% in the non-CAS group; n=5, 16.7% in the CAS group), and intraoperative complications were fewer in the CAS group (two exposures of orbital fat in the non-CAS group; no complications in the CAS group).

CONCLUSION: Computer navigational systems appear to serve as a valuable adjunct in preoperative planning and safe intraoperative dissection.

Endoscopic sinus surgery (ESS) is a treatment of choice for chronic rhinosinusitis (CRS), with an expanding role in the management of other sinus, orbit, and skull base diseases. Despite the availability of excellent nasal telescopes and high-resolution preoperative computed tomography (CT), major complications such as blindness, central nervous system (CNS) trauma, and even death still occur, because the ESS may be compromised by anatomic complexity or intraoperative bleeding.[1] Of late, computer-aided surgery (CAS) technology has permitted a direct comparison of the intraoperative anatomy with preoperative imaging information.[2] After a registration and calibration process, the surgeon may point to a specific structure with the CAS instrument and then view the location of the instrument tip on the CT image.[3] The use of CAS systems may allow for more precise dissections and greater rates of sinus patency outcomes and fewer complications.[2-4] We describe our experience with CAS as applied to intraoperative guidance and compare it with surgery without imaging guidance, in terms of safety of surgery, duration, complications, and outcome.

METHODS

We retrospectively studied 60 patients with CRS who underwent endoscopic sinus surgery between January 1, 2000 and 30 December 2006, including 30 patients who had conventional endoscopic sinus surgery and 30 patients who had endoscopic sinus surgery with use of the navigational system. The LandmarX system (Medtronic-Xomed) was used for the image-guided cases. The presence of extensive disease (stage III–IV nasal polyposis) was based on the Lund-Mackay endscopic grade system (polyps were scored as grade I when restricted to the middle meatus, grade II when they reached beyond the middle turbinate, grade III when they reached the inferior turbinate, and grade IV when filled the nasal cavity). Moreover, the total score was more than 12 in the Lund-Mackay CT scan classification system of CRS (The Lund-Mackay CT scan grading system relied on a score of 0-2, depending on the absence, partial or complete opacification of each sinus system, and of the ostiomeatal complex) . Patients who had external sinus surgery or who were not diagnosed as having chronic rhinosinusitis were excluded. Before surgery, a standardized CAS sinus CT scan (1 mm axial image) was obtained and transferred to the CAS workstation, where coronal and sagittal images, in addition to 3D-model reconstructions and a software-based CT review was completed.

After induction of general anesthesia, a headset that permitted attachment of a reference frame, with light-emitting diodes for optical tracking, was placed securely on the patient's head and registration and calibration was performed. All patients underwent endoscopic ethmoidectomy, endoscopic maxillary antrostomy, endoscopic sphenoidectomy, and endoscopic frontal recess exposure with frontal sinus suction clearance. The CAS probes were used frequently for confirmation of the surgical tool position. Usually we kept most of the patients in the hospital for one or two days, with discharge, and followed up after one week, when endoscopic debridement was performed. Patients were then followed up every two weeks until the healing process was complete, and then every two months with local treatment. The various parameters compared descriptively between the two groups of patients included, patient demographics, surgical details, operative times, the incidence of complications, and the outcome.

Table 1. CT scan findings in both groups.

	Non-CAS surgery (%)	CAS surgery (%)
Maxillary opacity	30 (100)	30 (100)
Ethmoid sinuses opacity	30 (100)	30 (100)
Frontal sinus opacity	16 (53.3)	26 (86.7)
Sphenoid sinuses opacity	19 (63.3)	29 (96.7)
Expansion effect	12 (40)	14 (46.2)
Bony wall erosion	7 (23.3)	12 (40)
Intraorbital extension	4 (13.3)	11 (36.7)
Extradural extension	3 (10)	11 (36.7)

CAS: Computer-aided surgery

RESULTS

The study population included 38 males (63.3%) and 22 females (36.7%), with a mean age of 28.2 years (range 11-63 years). All the patients underwent ESS, for a diagnosis of CRS in 40 patients (66.7%) and allergic fungal sinusitis (AFS) in 20 patients (33.3%). Surgery was primary in 37 patients (61.7%), and revision in 23 patients (38.3%). The clinical characteristics of patients in both groups were almost similar, with most of the patients presenting with nasal obstruction and nasal discharge hyposmia. Six (20%) in the CAS group had proptosis and one had diplopia; in the non-CAS group, seven (23.3%) had proptosis. Additional comorbidities included allergic rhinitis in 54 patients (90%), nasoseptal deviation in 38 (63.3%), and bronchial asthma in 20 (33.3%). Nasal polyps were graded III in 12 (40%) and IV in 24 (80%) patients in the non-CAS group, while in the CAS group, polyps were graded III in 12 (40%) and IV in 28 (93.3%) patients. Most patients had similar surgeries and pre- and postoperative management. Comparison of CT scan finding was performed (Table 1). The mean time of surgery was 3 hours and 26 minutes in the CAS group and 3 hours and 13 minutes in the non-CAS group. A higher recurrence rate was seen in the non-CAS group (11 patients, 36.7%) compared to the CAS group (five patients, 16.7%) in the postoperative period ranging from one to two years. Five patients (16.7%) from the non-CAS group needed revision surgery compared to two patients (6.8%) from the CAS group. Intraoperative complications did not occur in any of the patients in the CAS group and exposure of orbital fat occured in two patients in the non-CAS group. The incidence of postoperative complications was similar for both the groups. Two patients in each group had secondary bleeding; one was treated conservatively and other had to be controlled under general anesthesia. Two patients developed adhesions that were released under local anesthesia. Three patients from the CAS group reported persistent headache in the first postoperative week, which resolved completely with analgesics.

DISCUSSION

Otorhinolaryngologic surgery in the region near the skull base must be safe and thorough. Most complications occur if the surgeon is not aware of the exact position of his instrument. On account of that, interest in the use of image guidance systems in otolaryngology increased with the development of ESS.[3,4] At present, electromagnetic and optical systems are the most widely used because of their acceptable accuracy to within 2 mm or less, the freedom of head movement, and the ability to track a variety of instruments.[1,4-7] In our department, we use the LandmarX system (Medtronic-Xomed) and we are the first university in Saudi Arabia using this technology. The indications for use of the navigation system are usually extensive disease or previous surgery with poor anatomic landmarks.[8] The navigation system is used to reduce complication rates, completely remove the disease, and hasten the recovery after surgery.

CAS is most helpful in specific anatomic areas, especially in the frontal sinus,[8] the sphenoid and the sphenoethmoid regions, the residual ethmoid partition and disease, in skull base identification, and in orbital dehiscence or orbital surgery for optic nerve or orbital decompression.[2,3,9] We have many difficult cases that cannot be done safely without this technology (for example, in those who have had previous surgery with distorted anatomy or those with bone erosions, and intraorbital or extradural extension (Figures 1 and 2).

Hepworth and colleagues performed surveys in the US and found that the most commonly acceptable indications for CAS were revision surgery, in 84%, followed by frontal and sphenoid surgery; and they agreed that image-guided surgery should become the standard of care for functional endoscopic sinus surgery.[3,4,8] Olson and Citardi found that the most common diagnoses that indicated the use of CAS were chronic frontal sinusitis (92%), previous surgery (81%), sinonasal polyposis (48%), AFS (3%), chronic granulomatous fungal sinusitis (2%), cystic fibrosis (2%), and previous maxillofacial trauma (2%).[2] In our group, we found that chronic rhinosinusitis with sinonasal polyposis was the most common diagnosis (67%) and approximately 46% had frontal sinusitis in the CAS group, and 33.3% with AFS, because this sinonasal problem was common in our area. Revision surgery was required in 38.3%.

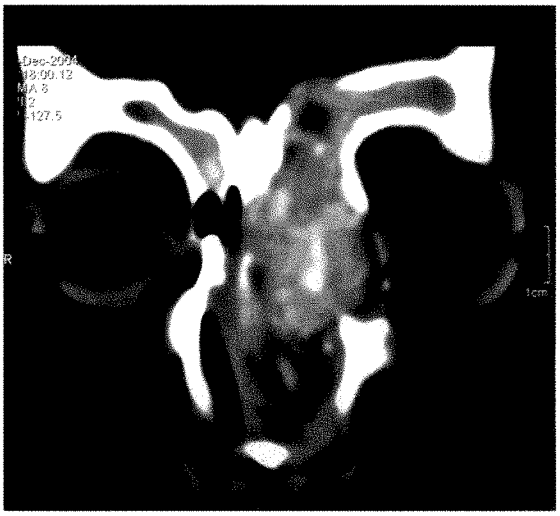

Figure 1. Fifteen years with allergic fungal sinusitis with intraorbital and extradural extension.

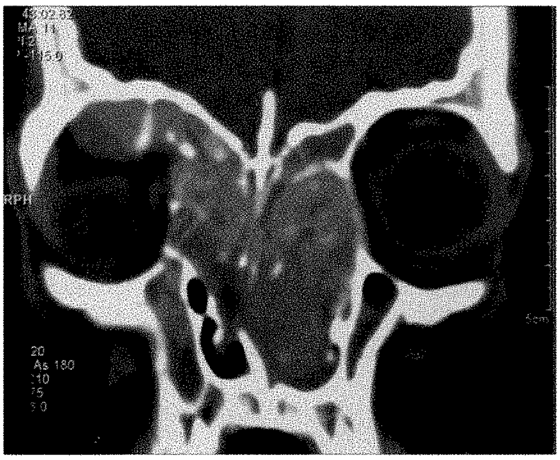

Figure 2. Sixteen years with supraorbital and intraorbital extension

Almost all of our patients underwent endoscopic ethmoidectomy, endoscopic maxillary antrostomy, endoscopic sphenoidectomy, and endoscopic frontal recess surgery. We found that CAS was valuable in confirming the surgeon's understanding of the paranasal sinus anatomy, and helpful in performing a more complete anatomic dissection in patients with extensive disease or heavy intraoperative bleeding, which helped to reduce intraoperative complications and provide better outcomes.[4,7] Also, we found it a valuable teaching tool in the residency training program in our institute.[3,7,10] Intraoperative CAS registration accuracy was estimated to be 2 mm or better.[1,6] To determine system accuracy, we compared the CAS instrument tip position indicated by the CAS system with its actual location in the operative field at several areas of the operative volume.[6] The estimated accuracy represents the surgeon's visual estimate of system accuracy.[7]

As outlined by Roth et al, CAS should provide an accuracy from 2 to 3 mm.[1,11] The system was used only if the perceived accuracy was <3 mm; if the drift was >3 mm the device was not to be used for localization. The device was verified at intervals ranging from 15 to

45 minutes.[3,9] In our group, we found the device was accurate up to 2 mm, by visually estimating the device probe to well-known visible anatomical landmarks. If it were not to this level, we reverified the device, to improve the accuracy. The duration of registration was approximately 15-30 minutes.

Initial use of the CAS has been found to increase the operative time by 15 to 30 minutes. Once a surgeon becomes familiar with the equipment, this time is typically reduced, from 5 to 15 minutes.[12] We have found that the operative time increases with CAS to approximately 10 to 20 minutes, with an average of 13 minutes, because of equipment manipulation and registration, but real time in surgery is reduced compared to non-CAS, because of improvement in the surgeon's confidence and knowledge of patient anatomy, which allows the surgeon to be more efficient while removing the pathology.[4,7,13]

Intraoperative image guidance is expected to increase safety and decrease complications during ESS.[2,6] When a surgeon is well versed in the sinus anatomy, in general, and in the individual patient's anatomy in particular, use of this technology to confirm the position within the sinuses, probably enhances the safety.[6,13] Major complications from sinus surgery are relatively infrequent.[1,2,14] The impact of image-guided technology on the complication rate has not been evaluated.[12]

In our patients, we found that the recurrence rate and complications were reduced even though the number of patients was not large enough to relatively demonstrate the outcome. In the CAS group, three patients complained of a persistent, moderate-to-severe headache, which was completely resolved with analgesics. The cause of the headache appeared to be due to the pressure caused by a tight-fitting headset. Wang and Maccabees reported six patients with facial neuropathies (both sensory and motor) related to the use of a headset.[15]

The future applications for image-guided surgeries are broad. They include otology, skull-base surgery,[13] and craniofacial surgery, as well as maxillofacial trauma.[7] In 1996, Fried et al. described the potential for using an MRI scanner for ESS in a suite that permitted 'real time' images and was set-up for operative procedures.[16] Klapan et al. designed a 3D-computer-assisted functional ESS for presurgical planning, intraoperative guidance, and postoperative analysis of the anatomic regions of the nose and paranasal sinuses.[14] Still, the technology is a promising avenue of research in the field of image-guidance for otolaryngology.[3]

In conclusion, the use of computer assistance is firmly established as a valuable technology in the management of paranasal sinus disease. It may improve the confidence of the surgeon by confirming the position within the challenging anatomic fields. This serves to increase surgical effectiveness and decrease surgical morbidity. The major drawback of using image-guided systems is the increased operative time, which improves with advancement in technology. The effect of CAS on the clinical outcome is yet to be demonstrated.

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case report

Homozygosity for the Mediterranean α-thalassemic deletion (hemoglobin Barts hydrops fetalis)

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Hemoglobin Barts hydrops fetalis syndrome is the most severe and generally fatal clinical phenotype of α-thalassemia. We diagnosed a fetus at 23-weeks gestation with having hydrops fetalis, by ultrasound. At 32 weeks, intrauterine death was detected. Molecular studies revealed that the fetus had the hemoglobin Barts hydrops fetalis syndrome due to homozygosity for the Mediterranean α-thalassemia deletion. This clinical phenotype is generally rare in the Eastern Mediterranean, and this is the first report of this syndrome from Iraq. Techniques for molecular characterization became available only very recently in this country, in a diagnostic setting. Thus, the detection of further cases might be expected in future.

Hemoglobin Barts hydrops fetalis syndrome, the most severe and generally fatal clinical phenotype of α-thalassemia, is due to the deletion of all four functional α-globin genes of the hemoglobin, resulting in no α-globin chain production. This clinical phenotype is generally rare in the Eastern Mediterranean. This is the first case report of molecularly confirmed hemoglobin Barts hydrops fetalis from Iraq.

CASE

A fetus was diagnosed with hydrops fetalis at 23-weeks gestation, by ultrasound, and intrauterine death was detected at 32 weeks; labor was induced and the fetus was delivered stillborn. The delivered stillborn had generalized edema, pallor, abdominal swelling, and hepatosplenomegaly. The placenta was large and friable. A full blood count performed on the cord blood using a Beckman-Coulter Hematology Analyzer (Fullerton, CA, USA) revealed a hemoglobin of 8.7 g/dL, a mean corpuscular volume of 94 fL, a mean corpuscular hemoglobin of 25 pg/cell, and a red blood cell (RBC) count of 3.46×10^{12}/L. The Leishman-stained blood film revealed RBC hypochromasia, marked anisocytosis, numerous tear drops and target cells, and large numbers of nucleated red cells (150/100 white blood cells).

The mother was 23-year-old and had only one pregnancy, two years previously. This also ended with the delivery of a stillborn hydropic child after 30-weeks gestation. A similar incident could not be recounted in either the maternal or paternal family histories. During her current pregnancy, the blood pressure was consistently normal, and serial prenatal examinations did not reveal any proteinuria. EDTA-blood samples taken from the mother and the 22-year-old father, who was her first cousin, were used to obtain blood counts, for hemoglobin electrophoresis on cellulose acetate (pH 8.6), and to measure hemoglobin A_2 by elution from cellulose acetate strips, and hemoglobin F by alkaline denaturation, using standard laboratory procedures.[1] Both parents also had their serum iron, total iron binding capacity, and transferrin saturation determined (BioMérieux-Marcy l'Etoile, France) (Table 1).

The blood samples from the couple and their hydropic stillborn were then used for DNA extraction using a phenol/chloroform method, and the DNA was amplified in a gap polymerase chain reaction as previously described,[2] to detect the Mediterranean ($--^{MED}$) α-thalassemic deletion. The amplification products were then run on 2% agarose gel (Figure 1).

Table 1. Some hematological parameters in parents of the hydropic child.

Parameter	Mother	Father
Hemoglobin (g/dL)	11.6	14.8
Hematocrit (L/L)	36.7	46.7
Mean corpuscular volume (fl)	63.3	67.9
Mean corpuscular hemoglobin (pg/cell)	20.0	21.5
Red cell distribution width (%)	15.9	15.7
Red cell count ($\times 10^{12}$/L)	5.79	6.88
Hemoglobin A2 (%)	1.5	1.9
Hemoglobin F (%)	2.0	1.4
Transferrin saturation (%)	20	26
Blood group	AB Rh(D) positive	AB Rh(D) positive

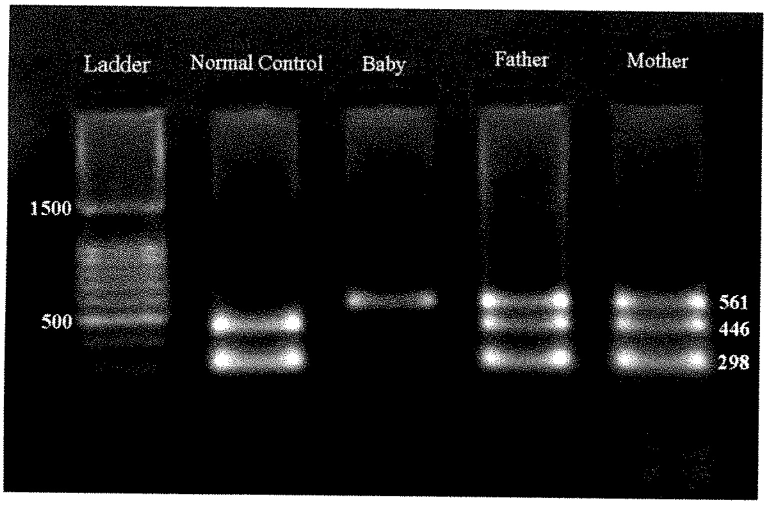

Figure 1. Gap-PCR for α-thalassemia deletions. The amplification products are run on 2% agarose gel. The normal (αα/αα) control shows two bands at 446 and 298 bp, while both parents show three bands (561, 446, and 298 bp) and are thus heterozygous for the Mediterranean deletion ($\alpha\alpha/--^{\mathrm{MED}}$). The infant shows only a single band at 561 bp and thus it is homozygous for the latter deletion ($--^{\mathrm{MED}}/--^{\mathrm{MED}}$).

Although both parents were heterozygous for ($--^{\mathrm{MED}}$) deletion (α-thalassemia minor phenotype), the stillborn child was homozygous for the deletion ($--^{\mathrm{MED}}/--^{\mathrm{MED}}$). This finding confirmed that the stillborn had hemoglobin Barts hydrops fetalis.

DISCUSSION

The hemoglobin molecule is a tetramer of two pairs of globin chains, each containing a heme group. During fetal life the main hemoglobin is hemoglobin F (α2γ2), while in normal adults the main hemoglobin is hemoglobin A (α2β2). The production of α-globin chains of hemoglobin is directed by two functional alpha genes (α1 and α2) located in the α-globin gene cluster at the short arm of chromosome 16.[3]

α-thalassemia is an inherited disorder of hemoglobin synthesis, characterized by reduced or absent α-globin chain synthesis.[4] It is caused by deletional or nondeletional mutations involving one (α^+ defects) or both (α^0 defects) alpha genes *in cis* at the α gene cluster[5]. Different combinations of these two defects result in four clinical phenotypes of α-thalassemia. The silent carrier state is caused by heterozygosity to the α^+ defect. α-thalassemia minor is caused by either homozygosity to the α^+ defects or heterozygosity to the α^0 defects. Hemoglobin H disease is caused by compound heterozygosity to the α^0 and α^+ defects, while homozygosity to the α^0 defect leads to the phenotype of hemoglobin Barts hydrops fetalis[3]. In the latter phenotype there is no production of α-chains, and therefore, the fetal hemoglobin (α2γ2) is not formed, but instead hemoglobin Barts is formed from four fetal γ chains (γ4). Hemoglobin Barts has a very high oxygen affinity, and will not be able to liberate oxygen at the physiological tissue tensions, and is thus ineffective as an oxygen carrier. Consequently, the fetus with this hemoglobin would have severe intrauterine hypoxia, leading to the intrauterine death of the hydropic fetus, late in pregnancy or at term.[4]

Several α^0 defects removing both alpha genes have been described in different populations, and they are named either in relevance to their geographical location or size. They include Southeast Asian ($--^{\mathrm{SEA}}$), Mediterranean ($--^{\mathrm{MED}}$), Philippino ($--^{\mathrm{FIL}}$), Thai ($--^{\mathrm{THAI}}$), and the 20.5 kb ($-(\alpha)^{20.5}$) variants.[4,6] Hemoglobin Barts hydrops fetalis is most common in Southeast Asian (SEA) countries, where it is mainly due to homozygosity to an SEA deletion ($--^{\mathrm{SEA}}/--^{\mathrm{SEA}}$).[6] It has also been reported, although uncommonly, in Mediterranean countries like Cyprus, Greece, and Turkey, where it is due to homozygosity to other types of α^0 defects, like the Mediterranean deletion ($--^{\mathrm{MED}}$) and the 20.5 kb α-thalassemia deletion ($-(\alpha)^{20.5}$).[7-9] Hemoglobin Barts hydrops fetalis, however, has not been reported, to our knowledge, in the Middle Eastern Arab countries, because of the scarcity of the Mediterranean deletion or other α^0 defects.[10,11] This report of hemoglobin Barts hydrops fetalis from Iraq is the first report from this country. It follows a recent report on the molecular basis of α thalassemia in northern Iraq, which documented that around a quarter of the α-thalassemia determinants were due to the Mediterranean deletion ($--^{MED}$).[12] Thus, it would not be unexpected that the couple who are both heterozygous ($\alpha\alpha/--^{MED}$), would be at risk of getting a fetus who is homozygous for this deletion ($--^{MED}/--^{MED}$).

Except for the most recent publication,[12] molecular defects responsible for α-thalassemia had not been previously studied in Iraq. Techniques for its molecular characterization only very recently became available in this country, in a diagnostic setting. Thus, it may not be unexpected to detect further cases of hemoglobin Barts hydrops fetalis in northern Iraq in the future, especially in view of the relative significant contribution of the ($--^{MED}$) deletions to α-thalassemia determinants in this part of the country. Accordingly, it maybe prudent to consider offering carriers of the Mediterranean deletion detected by premarital screening (currently practiced in this part of the country), the choice of prenatal diagnosis of this ultimately fatal condition by chorionic villus sampling at 10-12 weeks gestation.

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case report

Chronic anemia due to watermelon stomach

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Antral gastric vascular ectasia is a rare cause of chronic anemia. When encountered, the diagnosis is usually delayed. Endoscopic findings are well established, although radiologic findings are not. Patients respond well to surgery. Our case was of a 62-year-old female with chronic anemia who required multiple blood transfusions and iron replacement therapy, without significant response. Computed tomography revealed a focal thickening of the gastric antrum. Endoscopy showed vascular ectasia between the antrum and corpus. The patient underwent gastrectomy. We reviewed the literature on gastric angiodysplasia and have presented our unique tomography findings in this first report on a novel association between ectopic pancreas and gastric angiodysplasia.

Although the differential diagnosis of chronic anemia might seem straightforward, intriguing choices can be encountered during its evaluation. Finding the etiology of anemia can be challenging, especially in females and elderly patients. Evaluation starts with a basic physical examination, but may even require surgical exploration in difficult cases. The insidious nature of the chronic blood loss renders such patients symptomless for prolonged periods despite very low hemoglobin values. Rare disease associations and unusual clinical findings should be kept in mind for cases with obscure etiologies. We describe a rare cause of chronic anemia with a unique association.

CASE

A 62-year-old female patient presented to our outpatient clinic with fatigue. She had been followed for anemia, with changing hemoglobin values, between 7 and 11 g/dL (normal reference range, 12-18 g/dL), for over six years in different centers. An occult fecal blood test was positive and fecal parasite analysis was negative. She had received multiple tranfusions in the previous six years and had been started on oral iron replacement therapy before presenting to our clinic. Colonoscopy revealed normal findings. Two years previously she was diagnosed with antral vascular ectasia by endoscopy. Endoscopic cauterization of the bleeding foci had been performed twice.

At the latest presentation, a CT scan showed focal thickening of the gastric antrum (Figure 1). A repeat endoscopy revealed vascular ectasia between the antrum and corpus. Her hemoglobin level was 8.8 g/dL. The only serum biochemistry abnormality was a high indirect bilirubin of 1.55 (normal reference range, 0.10-1.20 mg/dL). Her physical examination was normal except for laparoscopic cholecystectomy incision scars.

Considering the intractable course of her disease, she underwent surgery. During the operation, the abdominal exploration was normal. After gastrotomy, columns of angiodysplasia were seen running from the antrum to the corpus (Figures 2 and 3). Hemigastrectomy and a Billroth 2 procedure were performed. The postoperative course of the patient was uneventful. She was discharged with normal oral intake.

The pathology report showed mucosal and submucosal malformation of the dilated vascular structures, which were compatible with angiodysplasia (Figures 4 and 5). A focus on ectopic pancreas was seen on the antrum.

DISCUSSION

Common causes of upper gastrointestinal hemorrhage include peptic ulcer disease, esophageal varices, gastritis, esophagitis, and Mallory-Weiss tears. One of the unusual causes is gastric antral angiodysplasia which results in major chronic gastrointestinal hemorrhage. Apart from anemia, gastric outlet obstruction is also reported in these patients.[1] The pathognomonic histological

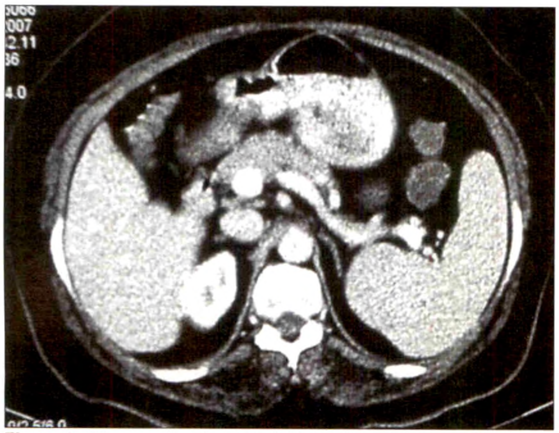

Figure 1. CT scan showing focal thickening of the gastric antrum.

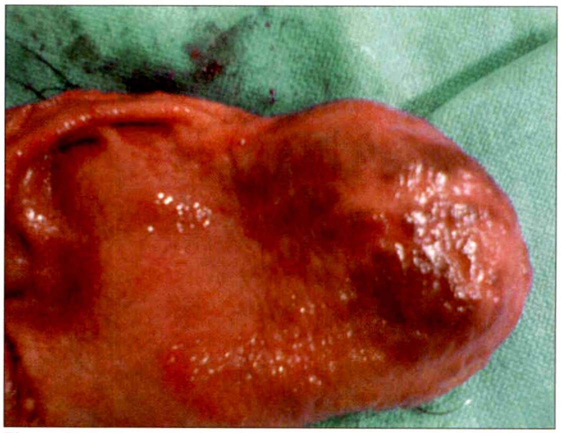

Figure 2. Columns of angiodysplasia.

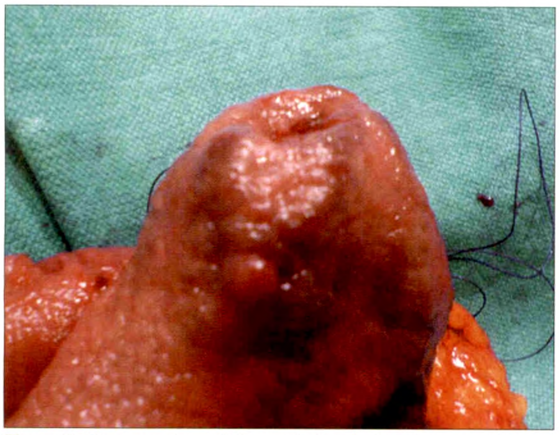

Figure 3. Close-up view of the angiodysplasia columns with antrum everted.

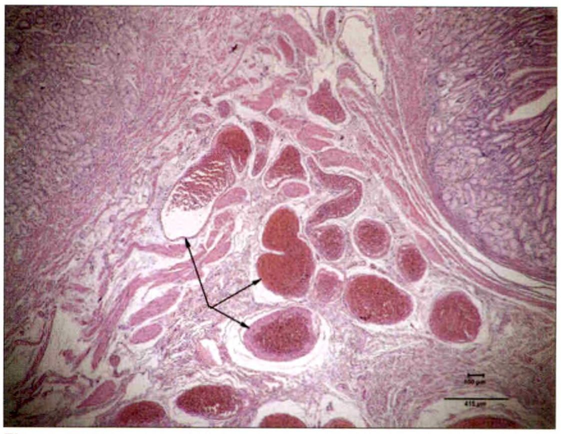

Figure 4. Submucosal abnormal vascular dilatation and structures that have penetrated into the mucosa, (hematoxylin and eosin, ×40).

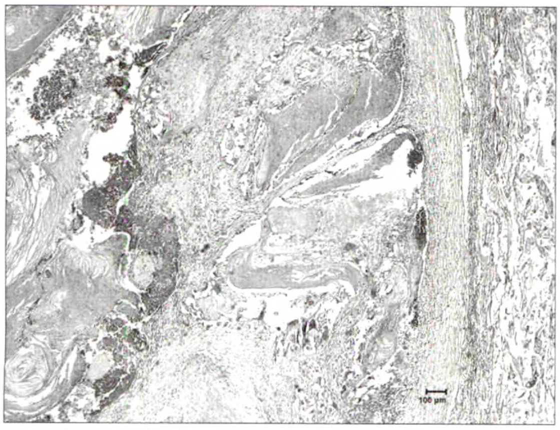

Figure 5. The lesion is well demarcated from the surrounding fibroconnective tissue, (hematoxylin and eosin, ×40).

features include foveolar hyperplasia,vascular ectasia with clots, and fibromuscular spindle cell hyperplasia of the lamina propria.[2,3] Chronic unexplained anemia resulting from bleeding of abnormal gastric vessels is a well recognized phenomenon but the condition is poorly documented and its etiology and pathogenesis are unclear.[4-6]

This syndrome was first recognized endoscopically by Wheeler et al. in 1979, but it was Jabbari et al. who first described three patients of their own and identified four cases from published reports.[5,7] The etiology of this condition remains unknown. These lesions can be seen in cirrhosis, in up to 30% of the patients, in two forms, as portal hypertensive gastropathy and gastric antral vascular ectasia, which are two seperate entities based on endoscopic and histolpathological findings.[8] In this setting, bleeding is usually slow and insidious and rarely massive and life threatening.[9-11]

In the absence of cirrhosis, gastric antral vascular ectasia is a distinct clinicopathological entity. The most widely accepted theory for the etiology focuses on the recurrent episodes of antral mucosal prolapse or an intussusception into the pylorus that would lead to chronic trauma and to consequent fibromuscular hyperplasia of the antral wall. Vasoactive substances as well as increased gastrin levels may play an important role in the pathophysiology, promoting vasodilatation and antral motility.[12] The resemblance of the histological findings to those of prolapse of the rectal mucosa, and those seen at stomal sites, support this theory.[3]

In the diagnosis, endoscopic findings are crucial and columns of dilated ectatic vessels on the summits of prominent antral rugal folds are characteristic. These appearances, however, can easily be misinterpreted as moderate-to-severe gastritis.[13]

Although recommended by most authorities, radiological investigations, including barium meal and mesenteric angiography, often fail to make the diagnosis, and identification of the cause of blood loss is usually delayed for many years.[14,15] These patients characteristically respond well to antrectomy and Bilroth anastomosis. Long-term corticosteroids and heat probe therapy have been reported as a safe options in older, medically unfit patients, while others have advocated endoscopic laser treatment.[16,17] Our case shows once again that in the workup for differential diagnosis of chronic anemia, gastric angiodysplasia should be investigated. Although there are not many reports on the characteristic CT findings in this entity, our case supports the CT findings of other authors. We feel that this should prompt a planned radiological research to identify characteristic appearances on CT, which could be used for patients not suitable for endoscopy. One other interesting finding was ectopic pancreas, in our pathology specimen. There are no other reports in the literature on this association.

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case report

Human infection with *Dicrocoelium dendriticum* in Turkey

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Human dicrocoeliosis is reported sporadically in various parts of the world. We report a case in a 21-year-old male, who had right upper abdominal pain, weight loss, and chronic relapsing watery diarrhea three to four times daily for four weeks. The patient had abdominal tenderness to palpation in the right upper quadrant. Alkaline phosphatase, alanine aminotransferase, and serum immunoglobulin E levels were slightly elevated; all other biochemical and hematological findings were in their normal ranges. The duodenal biopsy samples were normal and an abdominal ultrasonography showed no biliary or hepatic abnormality. Stool microscopy revealed numerous eggs of *Dicrocoelium dendriticum*. As pseudoparasitosis can result from eating raw, infected animal liver, the patient was given a liver-free diet for three days, to rule out that possibility. Subsequent stool examinations showed eggs in each of the samples indicating that the infection was genuine. The patient was treated with triclabendazole 10 mg/kg in a single dose. Four weeks later, no parasite eggs were detected in the microscopic examination of the stool samples. The patient got better gradually and the symptoms disappeared. Physicians should keep in mind parasitic diseases such as the rarely encountered dicrocoeliosis.

Dicrocoeliosis is caused by *Dicrocoelium dendriticum*, a 5 to 15 mm long and 1.5 to 2.5 mm wide parasitic fluke that usually infests the gall bladder and bile ducts of herbivores, such as, sheep, goat, cattle, and sometimes humans. The infection occurs as a result of oral ingestion of ants (belonging to the genus *Formica*, which is the second intermediate host) infected with metacercaria, via food.[1-3] Although spurious infections are sometimes encountered in stool examinations because of the ingestion of infected liver, real infections with this parasite are rarely reported in Turkey or elsewhere in the world in humans.[1-4] The infection is known to be widespread in ruminants in Turkey.[3]

Diarrhea lasting longer than four weeks warrants evaluation, to exclude a serious underlying pathology. In contrast to acute diarrhea, most of the causes of chronic diarrhea are noninfectious. However, some parasitic diseases may cause chronic diarrhea.[5] Although *D dendriticum* is less pathogenic than *Fasciola hepatica*, it leads to extensive damage in the liver by penetrating even to the most slender bile ducts. Accordingly, symptoms such as irritation in bile ducts, infectious hepatitis, a swollen abdomen, enlarged and painful liver, diarrhea, constipation, eosinophilia, and anemia are the symptoms also seen in fascioliosis.[1-3]

The prevalence of *D dendriticum* in our region is unknown. People living in the provinces generally only visit the hospital in cases of emergency. If an emergency occurs they prefer to first go to a small health center without equipment, where parasitological examinations are not usually available. This case is important because it attracts attention to parasitic diseases such as dicrocoeliosis, which are non-endemic and cannot be easily diagnosed clinically.

CASE

Our patient was a 21-year-old male who had right upper abdominal pain, weight loss, and chronic relapsing watery diarrhea three to four times daily for four weeks. On physical examination, the patient had abdominal tenderness to palpation in the right upper quadrant. There was no eosinophilia, and the erythrocyte sedimentation rate (ESR) was 14 mm/h. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), and serum immunoglobulin E levels were slightly elevated, (367 U/L (normal reference range, 0-270 U/L), 41 U/L (normal reference range, 0-41 U/L), and 253 U/mL (normal reference range, 0-100 U/mL), respectively. Direct and indirect bilirubin levels were in the normal range. Serologic tests for celiac disease were negative. There were no hormonal abnormalities causing watery diarrhea. Hepatitis B surface antigen was positive, but the HBV DNA level was low and the antibody to the hepatitis delta virus was negative. Furthermore, there were no signs of hepatic disease due to hepatitis B. All other biochemical and hematological findings were in the normal range. The duodenal biopsy samples were normal and the abdominal ultrasonography showed no bile duct or hepatic abnormalities. Stool microscopy of the patient revealed numerous eggs of *D dendriticum* (Figure 1), which were asymmetric, dark-brown, and had an operculated shell by wet smear in 0.9% saline solution and by the flotation method in saturated zinc sulfate. To confirm the result of the previous stool examination, new examinations were repeated consecutively over the next three days, after placing the patient on a liver-free diet, to determine whether the infection was real or psuedoparasitosis.

The eggs were seen in each of the samples, which indicated that the infection was genuine and not a spurious infection. In addition, it was also discovered that the patient had not eaten liver or liver products for the last seven days before the stool examinations were performed.

Following confirmation of the diagnosis, the patient was treated with triclabendazole (Fasinex®; Ciba-Geigy, Switzerland) 10 mg/kg in a single dose. Four weeks after treatment, stool samples of the patient were examined consecutively for three days using the same methods. The patient was almost free of symptoms and stool examinations for the parasite remained negative. The patient was completely treated with a single dose of the anthelmintic.

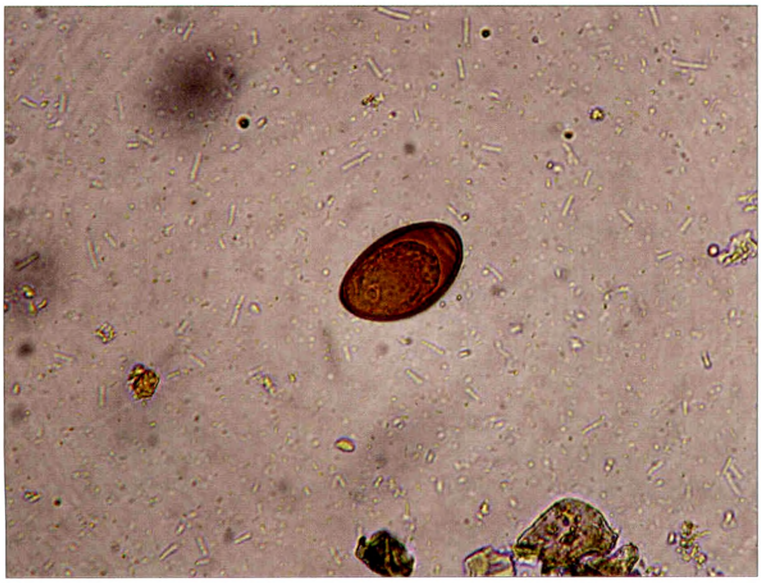

Figure 1. *D dendriticum* egg (42 µm × 26 µm) detected in the stool microscopy of the patient.

DISCUSSION

There are many causes of chronic diarrhea in humans.[5] Liver flukes are one of the causes of infection for chronic diarrhea.[1,2,4] Only a few dicrocoeliosis cases have been found among people in Turkey.[4,6-13] In the Cerrahpaşa Medicine Faculty Hospital (Istanbul University), dicrocoeliosis was detected in a 65-year-old female patient. The bile duct of the patient was obstructed with *D dendriticum*, and the patient was successfully treated with a single dose of triclabendazole (10 mg/kg).[12] At Kırıkkale University, surgeons operated on a 12-year-old girl with cholelithiasis due to *D dendriticum*.[13]

Cases of human dicrocoeliosis have been reported sporadically in various parts of the world.[14-18] In a study in Saudi Arabia, *D dendriticum* eggs were detected in 208 patients from 1984 to 1986, and at least seven of the 208 patients were diagnosed as having a real infection.[14] In another study in Saudi Arabia, true dicrocoeliosis was detected in 32 of 1196 patients by re-examination after three days of a liver-free diet.[15] In Germany, dicrocoeliosis was reported in a 36-year-old patient, who was successfully treated with triclabendazole (700 mg, single dose).[16] Another case of dicrocoeliosis reported in Germany, was of a 21-year old Afghani woman who had traveled to Germany as an immigrant.[17] In Czechoslovakia, the first case was reported in an 11-year-old boy in 1989.[18]

Although the parasite eggs have been detected high rates in stool samples in some studies in Turkey and around the world,[6-11,14,15] the researchers have not always clearly indicated whether infections were real; in some cases they may have been spurious. However, because experienced parasitologists are unavailable in some health centers, we think that some dicrocoeliasis cases may be overlooked.

The clinical symptoms of dicrocoeliosis are almost always the same as in fascioliosis. Although sometimes no symptoms are seen in patients infected with

D dendriticum, acute clinical symptoms such as eosinophilia, abdominal distention, painful liver, right upper abdominal pain, diarrhea, constipation, and anemia are observed in severely infected cases.[1] Our patient had only weight loss, watery diarrhea three to four times a day, and right upper abdominal pain for four weeks. Although his immunoglobulin E level was high, no eosinophilia or organomegaly was seen.

Triclabendazole is very effective in a single dose of 10 mg/kg, especially in the treatment of fasciolosis.[19-22] Therefore, we decided to use triclabendazole (Fasinex®; Ciba-Geigy) 10 mg/kg in a single dose for our patient. Following the treatment, stool examinations were performed by using the wet smear and flotation methods, for three consecutive days. No parasite eggs were detected in the stool samples, and the patient started to get better gradually and the symptoms began to disappear.

The real prevalence of *D dendriticum* in our region is unknown because people only go to the hospital when there is an emergency. If an emergency occurs they prefer to first visit village clinics, and often these clinics cannot perform parasitological examinations.

In conclusion, dicrocoeliosis can be associated with chronic watery diarrhea and right upper abdominal pain, and stool samples of the patients must be examined for intestinal parasitic diseases in health centers. Physicians should keep in mind parasitic diseases, even though they are encountered only rarely.

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case report

Successful transfer from insulin to oral sulfonylurea in a 3-year-old girl with a mutation in the *KCNJ11* gene

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Neonatal diabetes mellitus is considered a rare disease that is diagnosed in the first six months of life, and can be either transient or permanent. Recent advances in molecular genetics have shown that activating mutations in *KCNJ11* (the gene that encodes for the Kir6.2 subunit of the K_{ATP} potassium channel of the pancreatic β-cell) is a common cause of permanent neonatal diabetes mellitus. Patients with mutations in this gene may respond to oral sulfonyureas. We describe a 3-year-old girl with permanent neonatal diabetes mellitus with a mutation in the *KCNJ11* gene (R201H), who was successfully transferred from subcutaneous insulin to oral glibenclamide, with a marked improvement in glycemic control. This is the first successful switch from insulin to oral sulfonylurea in a patient with R201H mutation, in the Arabian Gulf.

Neonatal diabetes mellitus (NDM) is a rare disease with an incidence of 1 in 500 000 newborns. Neonates present with intrauterine growth retardation (IUGR) due to intrauterine insulin deficiency, glucosuria, polyuria, failure to thrive, and ketoacidosis, which usually appear in the first six months of life. Administration of insulin results in a dramatic improvement in the symptoms and growth.[1] NDM can present as permanent neonatal diabetes (PNDM) or transient neonatal diabetes mellitus (TNDM) that can sometimes be differentiated clinically. Molecular genetic analysis can dramatically differentiate between the two subtypes from the onset of the disease.[2] The common causes of PNDM are activating mutations in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the K_{ATP} sensitive channel of the pancreatic β-cell.[2] Mutations in *KCNJ11* cause PNDM in about 53% of the cases.[3] We report a case with a *de novo* heterozygous mutation in the *KCNJ11* (R201 H) gene that was successfully transformed from subcutaneous insulin to oral sulfonylurea. This mutation results in the inability of the K_{ATP} channel to close, in the presence of increased sensitivity of potassium channel (ATP). It has previously been observed that the introduction of sulfonyurea can close these channels by an ATP-independent mechanism.[4]

CASE

Our patient was 3 years old when she was diagnosed as having a de novo heterozygous mutation in the *KCNJ11* gene and transfered from subcutaneous insulin to oral glibenclamide. She was born at 40-weeks gestation, with a birth weight of 2 kg, to a healthy mother with no history of gestational diabetes. Her parents were consanguineous with no history of diabetes in the first- or second-degree relatives. At the age of 50 days she was admitted with an acute illness in the form of fever, vomiting, and diarrhea and she was found to have hyperglycemia (blood glucose >20 mmol/L) with no clinical or biochemical evidence of ketoacidosis. As her hyperglycemia was persistent she was started on subcutaneous insulin isophane NPH twice daily (0.3-0.5 units/kg/day). Her initial glycated hemoglobin (HbA1c) was 9% (reference range 4.4-

6.4%). Ultrasound of the abdomen showed the presence of pancreatic tissue. A skeletal survey was normal and liver function was normal. She was transferred to us for tertiary care at the age of two years where DNA molecular analysis was done for both parents and patient, after obtaining formal consent. At that time she was clinically well with normal development, and normal physical and neurological assessment. Her HbA1c then was 12% so she was changed to subcutaneous insulin glargine and rapid-acting analogs for better control. Her capillary blood glucose was measured four to six times per day (range of 15-20 mmol/L) with normal diet for her age. There was mild improvement in her HbA1c to 10-11% on changing her insulin regimen.

Genomic DNA was extracted from the peripheral leukocytes using standard procedures and the single exon of the *KCNJ11* gene was sequenced as previously described.[5] Sequencing of the *KCNJ11* gene detected a *de novo* heterozygous mutation in the *KCNJ11* gene (R201H) (Figure 1). At the age of three years the molecular genetic analysis showed that our patient had a *de novo* heterozygous mutation in the *KCNJ11* gene (R201H). The parents were informed and the child was admitted as an in-patient for transfer to oral sulfonylurea glibenclamide. The patient was transferred for a rapid in-patient transfer protocol.[3] Before starting glibenclamide, the physical examination and neurological assessment were performed, and they were normal for age. Regular capillary blood glucose monitoring was done four to six times a day, the blood was tested for ketones, the HbA1c was checked, and the usual daily dose of insulin was given prior to transfer. On the day glibenclamide was started, an oral glucose tolerance test was performed by giving glucose orally in a dose of 1.75 g/kg. After a sample of fasting blood glucose, insulin level, C peptide was obtained, followed by a postprandial glucose sample. After the oral glucose tolerance test (OGTT) a meal was allowed with rapid acting insulin and the first dose of glibenclamide was given, 0.1 mg/kg/dose, twice daily, in the form of a 5 mg tablet dissolved in water, at a concentration of 5 mg/ml. The following day's long-acting insulin was omitted; rapid acting insulin was continued as necessary, increasing glibenclamide by 0.2 mg/kg/day and continuing capillary blood glucose monitoring. She reached a dose of 0.8 mg/kg/day and insulin was weaned gradually until it was totally withdrawn after eight days of starting oral therapy. The transfer protocol was approved by our institute.

Our patient was almost three years old when the diagnosis of *de novo* heterozygous mutation in the *KCNJ11* gene (R201 H) was made. At that time she was switched to glibenclamide. Her height was 92.3 cm (twenty-fifth percentile for age), and weight was 14.7 kg (seventy-fifth percentile for age). Before transfer, her HbA1c was 10.7% (reference range 4.4-6.4%), fasting blood glucose was 5.6 mmol/L (reference range 3.3-5.6 mmol/L), the two-hour post-OGTT glucose was 20.1 mmol/L. Her blood glucose was monitored four to six times per day and was in a range of 15-18.6 mmol/L, with insulin of 150 pmol/L, while still on insulin (reference range, 12-150 pmol/L), and C-peptide <0.5 ng/mL (reference range, 1.1-5.0 ng/mL). She was weaned off insulin 8 days after starting glibenclamide, blood glucose monitoring was continued, with pre-prandial glucose in the range of 3.6-8.2 mmol/L and with midnight monitoring of glucose in the range of 3.6-10.8 mmol/L. Eight weeks later, while still on glibenclamide, with no episodes of hypoglycemia or diarrhea, capillary blood glucose was in the range of 5.5-8.0 mmol/L (decreased from previous recordings), HbA1c decreased from 10.7 to 7.1% (Figure 2), and the insulin level was 19 pmol/L. Twenty-four weeks from starting glibenclamide, she continued to show improvement. Capillary blood glucose monitoring, continued at home, showed that it was almost within the normal range and HbA1c dropped to 5.9%. The patient was compliant with the medications.

DISCUSSION

It has been shown that mutations in the *KCNJ11* gene are the most common cause of PNDM.[3] R201H is a mutation in the *KCNJ11* gene that usually presents in isolated diabetes, with no neurological phenotype.[5]

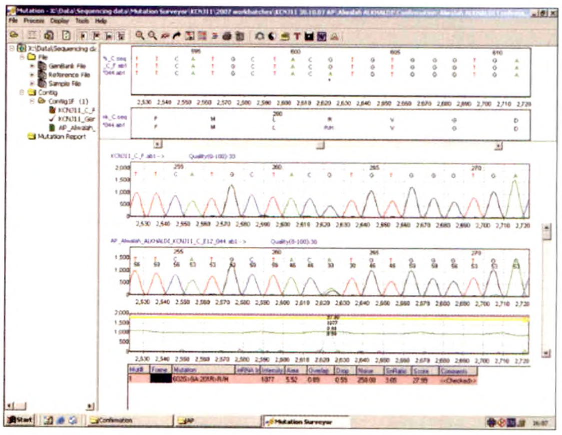

Figure 1. DNA sequence analysis of this patient revealed a de novo heterozygous mutation in *KCNJ11* gene (R201H).

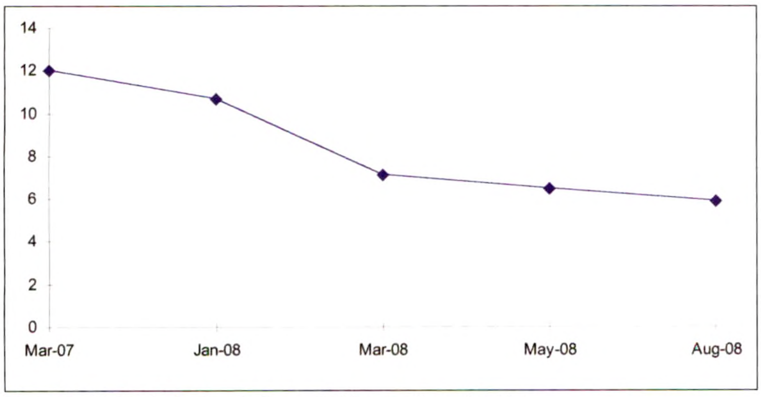

Figure 2. HbA1c before and after switching to glibenclamide.

Pearson et al showed the successful switch from insulin to oral sulfonyurea in 90% of patients with *KCNJ11* mutations.[4]

She had regular frequent follow up, eight months from the transfer she needed a reduced dose of glibenclamide (0.5 mg/kg/day), while still maintaining normoglycemic control as observed by Pearson et al.[4] Other mutations in *KCNJ11* (V59m) have been reported in Arab probands with an associated neurological phenotype,[7] but to the best of our knowledge, this is the first case of a successful switch from insulin to oral sulfonylurea in the Arabian Gulf, in a patient with R201H mutation. As the mutation in *KCNJ11* is clinically important and there is a proven response to sulfonyurea, it is mandatory to screen patients presenting with diabetes in the first six months, because it will have a major impact on the type of therapy, glycemic control, and quality of life. We have proven that switching from insulin to sulfonyurea was completely successful, without side effects, and the improvement in glycemic control was remarkable with results comparable to other case reports or group studies. However, long-term studies are needed to ensure the long-term safety and effectiveness of sulfonyurea.

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and supervise herbal shops with regard to the safety, preparation, storage, and dispensing.

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Congenital cutis laxa

PMID: 20220272

DOI: 10.4103/0256-4947.60528

To the Editor: Cutis laxa (CL) is a group of inherited and acquired disorders characterized by loose and redundant skin with reduced elasticity.[1] Autosomal dominant, autosomal recessive, and X-linked recessive patterns have been noted in the inherited forms.[2-4] The autosomal dominant form has a benign course; primarily, skin involvement is present, with few, if any, systemic complications, and a normal life expectancy. It can be caused by mutations in the elastin gene, but molecular heterogeneity cannot be excluded.[2,3] Type I autosomal recessive CL is characterized by pulmonary emphysema, umbilical and inguinal hernias, and gastrointestinal and vesico-urinary tract diverticula, and has the poorest prognosis. The Type II recessive form is called CL and is associated with joint laxity and developmental delay.[4] The histopathology of the skin in patients with CL reveals loss and/or fragmentation of elastic fibers.[5,6] All forms are very rare and no precise data about their prevalence are available.

We report an eight-year-old boy with congenital CL with umbilical and paraumbilical hernias, emphysema, and pulmonary artery branch stenosis. The child presented with fever and cough of seven days duration and four days of breathlessness of days duration. He was receiving treatment from a private practitioner, but was referred for increasing breathlessness. There was a history of recurrent episodes of cough and breathlessness since infancy, with an increase in the frequency and severity of the episodes in the past year. The patient had responded to oral medications in earlier episodes. This was the third child of nonconsanguineous parents with no similar family history. Early development was normal and the child was currently in the third grade and school performance was average. Increased laxity of the skin had been noticed six months of age, but a dermatology opinion was not sought. There was no history of skin rash or reaction to any drugs in the past. On examination, the child was febrile and had respiratory distress with intercostal and subcostal retractions. The respiratory rate was 88/min, heart rate 128/min, and blood pressure 100/70 mm Hg. There was no cyanosis or clubbing. The face had a senile appearance with an antimongoloid slant and slightly everted nostrils. The skin was loose and hanging in folds over the dorsum of the trunk and wrinkled over the face and on the dorsum of the hands. There was a left-sided, reducible, inguinal hernia and a small paraumbilical hernia (Figures 1 and 2); there was no laxity of joints. The weight and height were 20.5 kg and 118.5 cm, respectively, both at the fifth percentile for that age. Chest examination revealed an increased anteroposterior diameter with a Harrison sulcus. Rhonchi and coarse crepitations were heard bilaterally. There was

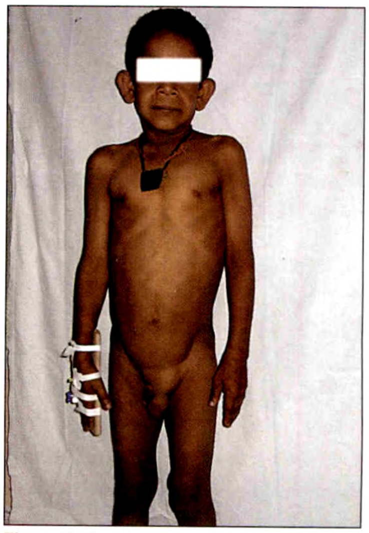

Figure 1. Senile appearance of the face, everted nostril, wrinkling over the dorsum of the hands, left-sided inguinal hernia, and small paraumbilical hernia.

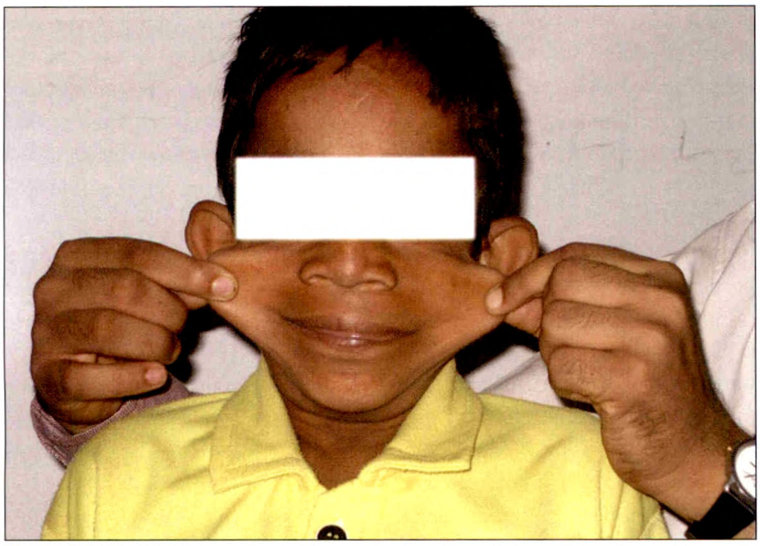

Figure 2. Laxity of skin.

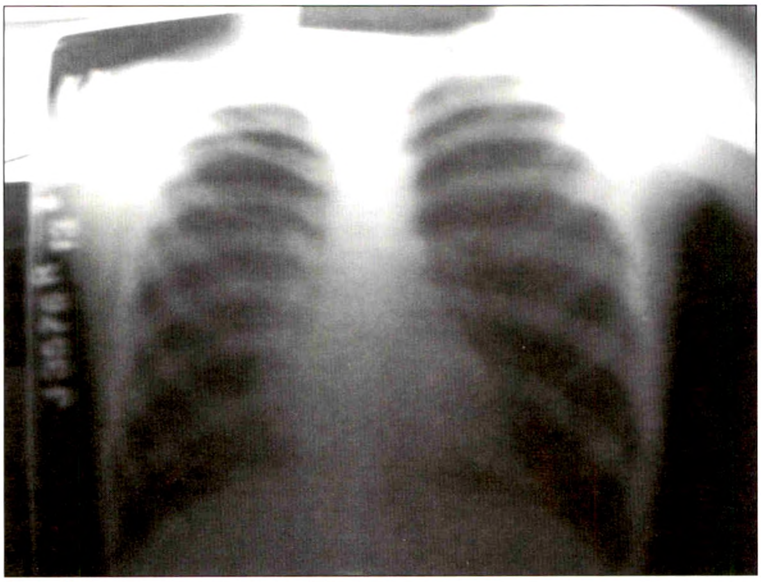

Figure 3. Chest x-ray showing emphysema.

no cardiomegaly; first and second heart sounds were heard normally. A Grade II ejection systolic murmur was heard in the pulmonary area. Examination of the abdomen revealed a palpable liver, 2 cm below the right costal margin, and a just-palpable spleen. Other systems were normal.

Tests showed a hemoglobin of 12 g/dL, a total WBC count of 7800/mm^3 with a differential count of polymorphs of 78%, lymphocytes of 20%, and eosinophils of 2%. A chest x-ray showed emphysema with patchy opacities in the right lower zone (Figure 3). Arterial blood gases showed no hypoxemia. There was no right ventricular hypertrophy on electrocardiography. Two-dimensional echocardiography showed stenosis of both the right and left pulmonary arteries. The child was treated with bronchodilators and antibiotics and was discharged after seven days. However, he was readmitted two weeks later for repair of his left inguinal hernia. Postoperative recovery was uneventful.

The diagnosis of a CL syndrome is based on clinical assessment of the typical skin features and the associated extracutaneous findings. In our patient, there was no history of any similar problem in any of the family members, thus ruling out an autosomal dominant inheritance. He had no history of developmental delay nor did he have any joint laxity, as described for Type II recessive CL; our patient probably suffered from the Type I recessive form. He had the characteristic cutaneous abnormalities described in all the varieties of CL. Additionally, he had paraumbilical and inguinal hernias, pulmonary emphysema, and stenosis of the right and left pulmonary arteries. Cardiopulmonary abnormalities are common in Type I recessive CL and are the main factors to determine the prognosis and life expectancy. Pulmonary emphysema, cor pulmonale, and right-sided heart failure caused by pulmonary disease have been commonly described. Various cardiovascular abnormalities including aortic aneurysm, pulmonary artery multiple branch stenosis, as in our patient, and pulmonary valve stenosis have been reported with this form of congenital CL.[1,7,8]

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RE: Extracorporeal circulatory systems in the interhospital transfer of critically ill patients: experience of a single institution

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To the Editor: The authors presented a therapy for patients with acute cardiopulmonary failure of gaining interest,[1] since several portable technologies for cardiopulmonary support have become available. However, the different technologies focus on different clinical indications and are suitable for different clinical strategies in providing extracorporeal cardiopulmonary support in emergency care. The aim of this letter is to highlight the key differences of the various systems and to indicate the most promising of these appealing new technologies that have the potential to markedly change the current treatment modalities for acute cardiopulmonary failure.

The clinical value of mechanical support as the optimal treatment for patients with acute circulatory failure currently is under intensive debate. As a result, current guidelines for the use of aortic counter pulsation in acute circulatory failure are questioned.[2] Advanced systems for mechanical circulatory support such as ECMO (extracorporeal membrane oxygenation) or percutaneously implantable heart-ling transplant systems have been established for emergency support of post-operative patients inside heart centers with circulatory or pulmonary failure.[3] These systems are ready for use after 30 to 45 minutes, require specialized technical personal for the set up as well as operation, are not qualified for patient transport, but are often designed to provide long-term cardiopulmonary support. Recently, short-term mechanical circulatory support more often is used in cardiogenic shock that can become manifest in a very rapid and often transient loss of circulation.[4,5]

With novel techniques of mobile, easy to use heart lung support systems like LIFEBRIDGE B2T (Lifebridge Medizintechnik AG, Ampfing, Germany) it is possible to provide full cardiopulmonary support within several minutes after the patients shows first signs of circulatory crisis.[6] The major advantage of this modular system (Figure 1) is that it can be applied very quickly and that it can be with access at any time and at any location where circulatory crisis occurs. A recent analysis of patients with acute coronary syndrome and cardiogenic shock by the "Euro Heart Survey" registry did find, that in the vast majority of events (71%) the circulatory crisis occurs in the hospital; the patient often does reach the hospital or already is hospitalized and "crashes" inside the hospital, where advanced health care such as circulatory support can be provided. In case of acute circulatory failure the rapid access of this emerging technology is of key value and it has been shown that it has a high impact on the survival of the treated patients.[7] The integrated safety features and the semi-automated priming sequence of the system allows trained personal on intensive care units, in the emergency room or cath lab to quickly regain sufficient circulation together with sufficient

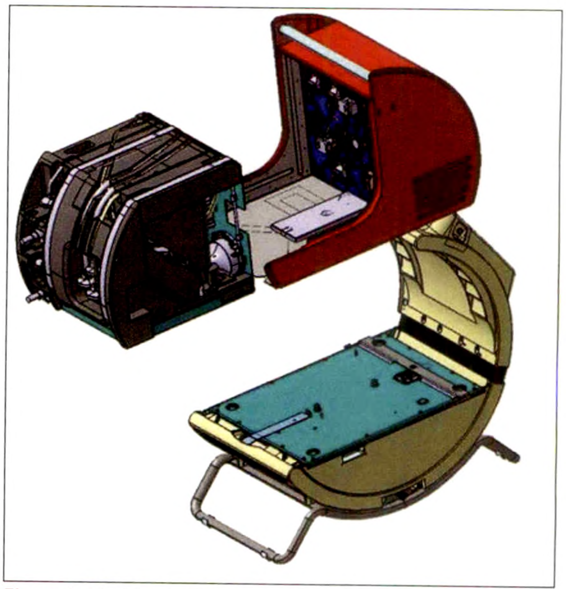

Figure 1. Modular configuration of the LIFEBRIDGE system: the base module (white), the stand alone, portable control module (red) and the patient module (grey, disposable).

oxygenation of the patient. Without any delay due to transfer time of equipment and specialized teams from heart centers, the patient quickly can be stabilized by the local team trained on using the mobile LIFEBRIDGE B2T system. De-airing and operation without risk of air emboli is guaranteed by a 7-step air management design that contains different active and passive processes to quickly establish and control extracorporeal cardiopulmonary support.[6] Within several minutes the system is primed and ready for use.

The cardiac surgeon no longer needs to get to the patient in circulatory crisis, since the rapid extracorporeal cardiopulmonary support can safely be established by a team consisting of an experienced interventional cardiologist or intensive care specialist who implants the femoral cannulas using Seldinger technique and a trained nurse that sets up the system in a semi-automated guided process of priming the system. This procedure serves as a powerful method of avoiding irreversible consequences of cardiogenic shock such as multi-organ failure or disease deterioration. After rapid normalization of arterial blood pressures and oxygenation a reasonable time window for the patient is opened. Extensive diagnostics including CT imaging can be performed under stable hemodynamic and metabolic conditions that lead to optimal organ protection. Once the diagnosis is made and no spontaneous recovery is achieved, the underlying disease can be treated (for example, surgery, high-risk percutaneous coronary intervention), if required under extracorporeal support.

In case of other treatment options patients can safely be transferred to remote hospitals with specialized treatments such as transplant programs or assist programs. Currently, the ECLS (extracorporeal life support or ECMO transports usually are very complex and guidelines for staff and patient safety during transport are not met. However, the Conformité Européenne-marked emergency cardiopulmonary support systems LIFEBRIDGE B2T system is the first and only HLM approved to date for mobile use according European norm EN 1789. With the availability of these novel systems, the clinical use of extracorporeal circulation outside the operating room to treat circulatory or pulmonary failure is expected to increase rapidly in the future, and unaddressed clinical needs in acute intensive care will be met.

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The author declares no conflict of interest.

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Type V aplasia cutis congenita

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A two-hour-old female neonate, a product of nonconsanguineous marriage with unremarkable family history, was brought with well-defined bilaterally symmetrical and superficial erosions on the knees, trunk and lower limbs with sparing of scalp and mucosa (Figure 1). Biopsy revealed an absence of epidermis and superficial dermis. Antenatal records revealed that the mother was diagnosed with a twin pregnancy at 12 weeks gestation with a follow-up scan revealing pregnancy loss (fetus papyraceous). There was no maternal history of drug intake. There were no other congenital malformations. Ultrasonography of the head and abdomen and karyotyping and screening for intrauterine infections were normal. The child was managed conservatively with paraffin gauze dressings. The lesions evolved into fibrous scars (Figure 2). No new skin lesions appeared. A diagnosis of type V aplasia cutis congenita (ACC) with fetus papyraceous was made.

Type V ACC is a nonscalp cutis aplasia with fetus papyraceous.[1] It results from temporary hypotension in the surviving twin at the time of fetal demise in the early second trimester leading to poor perfusion and skin necrosis.[2] The lesions of type V ACC are typically bilaterally symmetrical. The lesions are noninflammatory, well demarcated and vary in size from 0.5 to 10 cm. Diagnosis is based on the physical appearance of the skin and biopsy. The appearance of the lesions and histological features at birth vary depending on when they occur during intrauterine development and depth of aplasia. Lesions that form early in gestation may heal before delivery and appear as fibrotic scars, whereas less mature defects may present as an ulceration of variable depths (erosions/ulcers). ACC

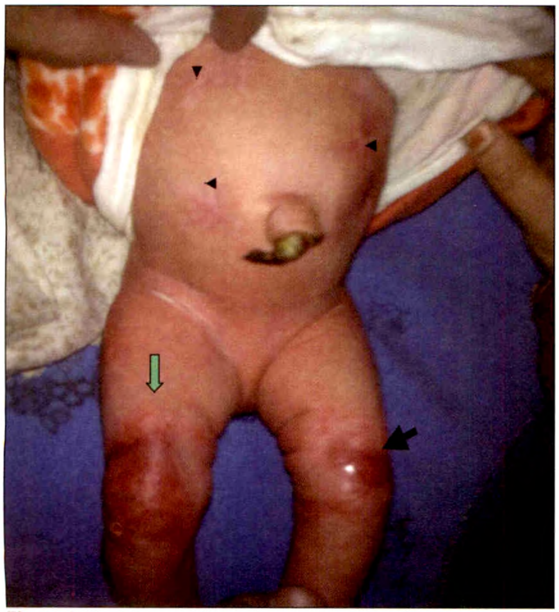

Figure 1. Translucent blister (green arrow), well demarcated superficial skin erosion (black arrow), and initial stages of scar formation (arrowheads).

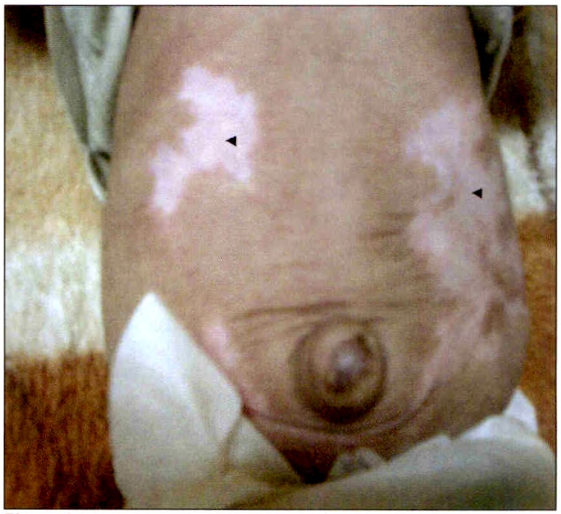

Figure 2. Atrophic pale fibrous scars (arrowheads).

must be differentiated from the epidermolysis bullosa (mucosal involvement), Transient bullous dermolysis of the newborn resolves during the first months of life leaving no dystrophic scars. Treatment includes gentle cleansing and applying bland ointment or silver sulfadiazine ointment.

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links for authors and reviewers

Good reporting of trials and observational studies: Related guidelines and initiatives

EQUATOR

EQUATOR <http://www.equator-network.org> (Enhancing the QUAlity of Trials and Other Research) The EQUATOR Network is a new initiative that seeks to improve the quality of scientific publications by promoting transparent and accurate reporting of health research.

STROBE

STROBE **(STrengthening the Reporting of OBservational studies in Epidemiology)** is an international, collaborative initiative of epidemiologists, methodologists, statisticians, researchers and editors involved in the conduct and dissemination of observational studies.

CONSORT (Consolidated Standards of Reporting Trials), is part of a broader push to produce reporting guidelines (RGs) for many different types of research. The main product of CONSORT is the CONSORT Statement, which is an evidence-based, minimum set of recommendations for reporting RCTs. It offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation. The CONSORT Statement comprises a 22-item checklist and a flow diagram, along with some brief descriptive text.

ASSERT

The ASSERT **(A Standard for the Scientific and Ethical Review of Trials)** initiative is a proposed standard for the review and monitoring of randomized clinical trials by research ethics committees. Among other items, its checklist incorporates certain elements of CONSORT, to ensure fulfillment of the requirements for scientific validity in the ethical conduct of clinical research.

MOOSE

The MOOSE **(Meta-analysis of Observational Studies in Epidemiology)** paper reports the outcome of a 1997 workshop to examine the reporting of meta-analyses of observational studies. It makes recommendations to aid authors, editors, reviewers and readers of observational research.

QUOROM

The QUOROM **(Quality Of Reporting Of Meta-analyses)** conference resulted in the creation of the QUOROM Statement, which consists of a checklist and flow diagram.

STARD

The STARD **(Standards for Reporting of Diagnostic Accuracy)** initiative aims to improve the accuracy and completeness of reporting of studies of diagnostic accuracy in order to allow readers to assess the potential for bias in a study and to evaluate the generalisability of its results.

King Faisal Specialist Hospital and Research Centre

Symposium:

Annual Saudi Colorectal Surgery Forum
27-31 March 2010
11-15 Rabi Al Thani 1431

3rd Mini AABB Symposium
30 March–01 April 2010
14-16 Rabi Al Thani 1431

International Symposium on New Frontiers in Breast Cancer
27-29 April 2010
13-15 Jumada Al Awal 1431

Academic & Training Affairs
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World Health Organization
Regional Office for the Eastern
Mediterranean

Directory of Health, Medical Conferences and Professional Meetings in the Region

http://www.emro.who.int/conf/

Objectives

To develop and maintain a directory of conferences and meetings organized by government institutions, academic bodies and other non-profit professional organizations in the Region.

The directory will:
- Serve as a vehicle to distribute information on these events to health care professionals and academicians;
- Enhance networking and collaboration between institutions;
- Record professional events for the future reference and archiving of conference proceedings as much as possible;
- Alert health care professionals and academicians to the new conferences related to their speciality.

Section of Colorectal Surgery Fellowship Training Program

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announcements

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Dr. Alaa Abduljabbar,
Chairman, Organizing Committee
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مخططات الوزن السوية للجنين في الحمل المفرد لدى السكان الأردنيين

مقدمة: إن تقدير وزن الجنين في الرحم (EIUFW) ضروري للتقليل من معدل الوفيات والمراضة قبل الولادة من خلال الاكتشاف المبكر لاضطراب النمو. كان هدفنا هو الكشف عن أنماط وزن الجنين التقديري في الرحم بالموجات فوق الصوتية من خلال تحديد عمر الحمل للحمل المفرد الطبيعي وتقييم تأثير عدة متغيرات على وزن الجنين التقديري في الرحم (EIUFW).

المرضى والطرق: قمنا بتحديد وزن الجنين التقديري في الرحم (EIUFW) لدى ٦٠٠ امرأة حامل في الأسبوع الـ ٢٠ إلى ٤٢ من الحمل (WG). وقمنا بتصنيف وزن الجنين التقديري في الرحم (EIUFW) إلى منخفض أو طبيعي من خلال استخدام الشريحة المئوية العشرة (١٠) والعشرون (٢٠) كنقاط فاصلة. كما استخدم تحليل التراجع اللوجستي لحساب نسب الاحتمال نطاق الثقة ٩٥٪ لعدد من العوامل الخطيرة التي يفترض أن تكون مترابطة مع انخفاض وزن الجنين. كما تم حساب النسب المئوية (٢٥، ٥٠، ٧٥) لوزن الجنين التقديري في الرحم من خلال عمر الحمل والجنس للحمل المفرد.

النتائج: لم نجد فارقاً كبير من الناحية الإحصائية بين الأجنة الذكور والإناث فيما يتعلق بوزن الجنين التقديري في الرحم (EIUFW) حتى الأسبوع الثاني والثلاثين من الحمل. ووجد ارتفاع ملحوظ في أوزان الأجنة الذكور (*P*<.05) عن الإناث بين الأسابيع الـ ٣٢ إلى ٣٩ من الحمل. وقد تم إنشاء جداول وزن الجنين التقديري في الرحم (EIUFW) بالموجات فوق الصوتية من خلال عمر الحمل والجنس للحمل المفرد. ولاحظنا ترابط وزن الجنين التقديري في الرحم (EIUFW) مع مجموعة من المتغيرات مثل زيادة الوزن في الحمل، ومستوى الهيموجلوبين لدى الأمهات، وتواتر الزيارات قبل الولادة، وحالة التدخين، ونوع الجنين.

الاستنتاج: حين تقل زيادة الوزن لدى الحوامل عن كيلوجرام واحد شهرياً في الثلثين الثاني والثالث من الحمل يتوجب تشجيع الأمهات الحوامل على زيادة أوزانهنّ خلال فترة الحمل. وهناك حاجة لإجراء دراسة استطلاعية على شريحة وطنية ممثلة في الأردن بهدف تحديث معاييرنا الخاصة بنمو الجنين.

الفروق في قراءة المختصين بالأشعة في الكشف عن التغيرات الرئوية المتنية الشاذة على التصوير الطبقي المحوسب رفيع الميز

مقدمة: تتأثر التقييمات الشعاعية والنسجية بالتفسير الشخصاني. حددت هذه الدراسة مستوى الفروق في قراءة المختصين بالأشعة في الكشف عن التغيرات الرئوية المتنية الشاذة على صور التصوير الطبقي المحوسب رفيع الميز.

المرضى والطرق: تم اجراء دراسة استعادية لصور طبقية محوسبة رفيعة الميز لـ ٦٥ مريضاً من مرضى الذأب الحمامي المتعمم (مع تأثر رئوي سريري) على يد أربعة من اختصاصيي الأشعة غير الصدرية (إثنان تصوير بالرنين المغنطيسي. وإثنان تصوير عام). قام كل إختصاصي بقراءة النتائج مرتين. فصل بين الواحدة منها والأخرى مدة لا تقل عن ٦ شهور. وتم تقييم فرق الملاحظة البيني للقراءة الأولى والثانية للإختصاصيين الأربعة. وفرق الملاحظة الفردي لقراءتي كل اختصاصي باستخدام طريقة احصاء كابا kappa statistic.

النتائج: كان هناك توافق جيد بين القراءتين الأولى والثانية لكل اختصاصي أشعة. وكان هناك توافق متوسط بين قراءتي احد اختصاصيي التصوير بالرنين المغنطيسي (كابا = ٠,٤٨٢). وتراوحت قيم كابا للإختصاصيين الثلاثة الآخرين من جيد إلى ممتاز (٠,٧١٦ ، ٠,٦٩١ ، و٠,٨٢٩). كان هناك مستوى سريري مقبول للفروقات البينية للإختصاصيين الأربعة. كان الإتفاق من مقبول إلى متوسط بين اختصاصيي التصوير بالرنين المغنطيسي والمراقبين الآخرين (مدى كابا ٠,٣٦٢–٠,٥١٩). ومتوسط إلى جيد بين اختصاصيي الأشعة الثلاثة الآخرين (٠,٥٠٨–٠,٧٣٠).

الاستنتاج: إن تفسير موجودات التغيرات الرئوية المتنية الشاذة على صور التصوير الطبقي رفيع الميز متناغ بالتكرار، ومستوى التوافق بين اختصاصيي الأشعة العامة مقبول سريرياً. ويقل هذا المستوى مع تباين التفسير بين اختصاصيي الأشعة غير المعنيين بتصوير الصدر بصفة منتظمة. قد تستفيد الحالات الصعبة أو غير محددة المعالم من مراجعة يجريها اختصاصي بالأشعة الصدرية.

تحليل دراسات منهجية للعلاج شديد الفعالية المضاد للفيروس القهقري في معالجة أورام أرومة الخلايا البلازمية

مقدمة: وصف ورم أرومة الخلايا البلازمية مؤخراً بأنه ورم لمفاوي مشتق من الخلايا البائية. يكون التكهن بمآل المرضى الذين يعانون من ورم أرومة الخلايا البلازمية سيئاً. أجرينا مراجعة منهجية على ما نشر عن استخدام العلاج شديد الفعالية المضاد للفيروس القهقري (HAART) والتكهنات بمآل لمفوم أرومة الخلايا البلازمية.

الطرق: أثمرت نتيجة البحث الشامل لقواعد البيانات ذات الصلة بالموضوع. والتي شملت Medline، Embase. سجل Cochrane Controlled Trials Register. مكتبة Cochraneوجدول التنويه العلمي Science Citation Index. عن عشرة تجارب عشوائية منضبطة. وقد قُسمت التجارب إلى مجموعتين حسب نوع المعالجة المستخدم. وتم تحليل معدلات الأورام أرومية الخلايا البلازمية باستخدام نماذج تأثير ثابتة. وتحليل الحساسية (نموذج إحصائي) التي أجريت لاكتشاف المزيد من الحالات وتقييم التغير الواضح. وقد تم إجراء فحوص نسبة البقيا لدى من يعانون من الورم الأرومي اللمفاوي البلازمي باستخدام أسلوب Kaplan-Meier.

النتائج: أظهرت نتيجة التحليل المنهجي اختلاف المآل إحصائيا لدى المرضى الذين يعانون من الورم الأرومي البلازمي ممن يتلقون العلاج شديد الفعالية المضاد للفيروس القهقري HAART بالإضافة إلى المعالجة الكيميائية و/أو المعالجة الإشعاعية مقارنة بالمرضى الذين يتلقون المعالجة الكيميائية و/ أو المعالجة الإشعاعية فقط(احتمال الخطورة النسبي=٣،٠٤; P=0.03). وقد أظهر تحليل معدلات البقيا أيضاً وجود اختلاف إحصائي هام (c^2=6.22,P=.013).

الاستنتاج: يعد استخدام العلاج شديد الفعالية المضاد للفيروس القهقري بالإضافة إلى المعالجة الكيميائية و/ أو المعالجة الإشعاعية فعالاً للتحسين من مآل الورم الأرومي البلازمي. وبالرغم من ذلك، فإن الأحجام الصغيرة للعينات تزيد من احتمالات انحياز الدراسات في هذا التحليل. لذا يجب أخذ هذه النتائج بقدر من الحيطة.

الارتباط وتحليل النمط الفرداني لمستقبلات المنشطات الكيماوية المتجمعة على الصبغي ٣p٢١,٣ والتي تُتوارث في شجرة العائلة لدى الذين يعانون من الربو والتأتب الوراثي

مقدمة: يشير تحليل المسح الجينومي إلى أن مجموعة مستقبلات المنشطات الكيماوية (CCR2, CCR3, CCR5 <300 kb span) على الذراع القصيرة للصبغي ٣ قد تساهم في قابلية الإصابة بفيروس نقص المناعة البشري-١ وعدد من الأمراض الالتهابية. كما تم العثور على اثنين من تعدد أشكال النوكليوتيد المفرد (SNPs) وخبن في مستقبلات المنشطات الكيماوية من خلال دراسات الحالات والشواهد في قابلية الإصابة بالربو والأنماط الظاهرية ذات الصلة. وقد قمنا بتمديد دراسات الحالات والشواهد عبر تقرير ما إذا كان تعدد الأشكال مترابطاً واختلال توازن الترابط مع الربو والأنماط الظاهرية ذات الصلة باستخدام تحليل الترابط والنمط الفرداني.

المرضى والطرق: قمنا بتحديد النمط الجيني لـ ١٥٤ أسرة بيولوجية تم تعريفها من خلال طفلين مستلفتَين أصليين بتشخيص طبي للربو (٤٥٣ شخصاً لا تربطهم صلة) بما في ذلك ٣٠٣ من الآباء التي لا تربطهم صلة و١٥٠ طفلاً لا تربطهم صلة. تم تعريف التأتب الوراثي بايجابية (٣ ملم) اختبار وخز الجلد (SPT) لمجموعة من المواد المؤرجة المستنشقة.

النتائج: من بين ١٠ لائحة تضم عشرة أشكال متعددة معروفة للنوكليوتيد الفرداني (SNPs)، تبين ظهور ثلاثة شذوذات بتكرار ذي مغزى سريري اثنان من تعدد الأشكال. G190A- في CCR2، T51C- في CCR3، وخبن 32-BP في CCR5. تمت الاستعانة بـ ١٥٤ أسرة من أجل تحليل النمط الفرداني، ولكن ١٢ أسرة بيولوجية كانوا مؤهلين فقط لتحليل الترابط. أكدت كلا التحليلات بأن الطفرات مرتبطة بالربو وليس بالتأتب الوراثي.

الاستنتاج: تعتبر جينات مُستقبل المنشطات الكيماوية على الصبغي ٣p٢١,٣ مرشحة بشكل واضح في إثارة الربو. إن الترابط السابق بين خبن CCR5 d32 مع الحماية من الإصابة بالربو في مرحلة الطفولة يبدو مفسراً عبر اختلال توازن الترابط مع طفرة G190A في المُستقبِل CCR2.

الأنواع الجزئية للنمط الجيني الرابع لجينوم فيروس التهاب الكبد ج لدى المرضى السعوديين الذين يتلقون معالجة بواسطة انترفيرون وريبافيرين

مقدمة: لا يستجيب العديد من المرضى المصابين بفيروس التهاب الكبد ج للمعالجة المضادة للفيروسات، وذلك على الأغلب بسبب الأنواع الجزئية للفيروس. لذا قمنا بالاستقصاء بشأن إمكانية استخدام جمهرة الأنواع الجزئية للتكهن باستجابة مرضانا للمعالجة.

المرضى والطرق: تمت دراسة الأنواع الجزئية للنمط الجيني الرابع لفيروس التهاب الكبد ج عند ٢٥ من المرضى السعوديين حديثي العهد بالعلاج بعد تلقيهم معالجة بمزيج من انترفيرون ألفا (IFN-alfa) وريبافيرين مباشرة، وبعد المعالجة بثلاثة شهور، وبعد المعالجة بستة شهور، وتم تحليل المنطقة الأولى عالية التغيير ضمن الجين NS١/E٢ من الفيروس بواسطة طريقة تعدد هيئة الطاق المفرد (SSCP) بعد التضخيم.

النتائج: تم اكتشاف أشرطة الدنا في جميع المرضى قبل المعالجة بطريقة SSCP (٢-٧ شريط). ولدى الذين حققوا استجابة فيروسية تامة خلال ستة شهور (الحمولة الفيروسية ›٠.٢ ملي مكافئ /لتر: n=٧). تراوح عدد الأشرطة [متوسط ٢-٦: ٣.٧١ (١.٢٥)]. وفي ستة من المرضى السبعة، فإن عدد أشرطة SSCP بقي بدون تغيير أو نقص العدد تسلسلياً. أما بالنسبة للمرضى الذين لم يستجيبوا (الحمولة الفيروسية ‹٠.٢ ملي مكافئ /لتر: n=١٨)، فإن عدد الأشرطة تراوح أيضاً بين ٢-٧: متوسط ٣.٧٧ (١.٧٣). وفي ستة من المرضى الذين لم يستجيبوا، بقيت أشرطة SSCP كما هي أو نقصت تسلسلياً. لم يكن هناك فرق هام بين تركيب الأنواع الجزئية قبل المعالجة والاستجابة (P=٠.٥٣). استجاب اثنان من أربعة مرضى كان لديهم حمولة فيروسية عالية قبل المعالجة بنفس تركيب أشرطة الأنواع الجزئية أو أقل.

الاستنتاج: لا يمكن استخدام الأنواع الجزئية لدى المرضى الذين أجريت عليهم الدراسة للتكهن بمدى الاستجابة للمعالجة، ولكنها قد تشرح سبب عدم استجابة معظم المرضى المصابين بالنمط الجيني الرابع لفيروس التهاب الكبد ج للمعالجة بانترفيرون ألفا وريبافيرين.

الاستعمار بالمبيضات: تنوع السلالة والحساسية للمضادات الفطرية لدى مرضى السكري البالغين

مقدمة: يعد الاستعمار بالمبيضات لدى مرضى السكري موضع نقاش. الهدف من هذه الدراسة هو استقصاء الاستعمار بالمبيضات في الفم وتنوع السلالة والحساسية للمضادات الفطرية وأثر العوامل الموضعية والجهازية لدى المضيف على الاستعمار بالمبيضات لدى مرضى السكري البالغين.

المرضى والطرق: أجرينا دراسة شاهدة للحالات حيث قمنا بمقارنة ١٥٠ مريض بالسكري (٤٩ منهم يعانون من النوع ١، و ١٠١ منهم من النوع ٢) مع ٥٠ مريضاً شاهداً من الأصحاء. تم جمع عينتي لعاب: إحداها لتحديد معدل تدفق اللعاب ودرجة الحموضة pH . أما الثانية فقد جمعت لتقييم الاستعمار بالمبيضات باستخدام طريقة المضمضة لجمع العينات. تم التعرف على معزولات المبيضات وفحصها في مختبر حساسية المضادات الفطرية باستخدام العتائد التجارية Candifast. كما تم استقصاء العلاقة بين عوامل لدى المضيف معينة والاستعمار بالمبيضات.

النتائج: لدى مرضى السكري معدلات أعلى لحمولة المبيضات لكن ليس بنسبة الكثافة. بالمقارنة مع الحالات الشاهدة. ووجدنا أن المبيضات البيضاء من أكثر أصناف الفطريات الموجودة لكن وجدنا لدى مرضى السكري تنوعاً في أصناف المبيضات. ولم تقاوم العينات من المجموعة الشاهدة أية مضادات فطرية تم فحصها. بينما أظهرت عينات مرضى السكري مقاومة متباينة للمضادات الفطرية من فئة آزول. في حين وجدنا ترابطاً إيجابياً واضحاً بين انضباط نسبة السكر في الدم والاستعمار بالمبيضات في داء السكري النوع ٢. بينما كان الترابط سلبياً بين عينة حموضة اللعاب pH وحمولة المبيضات في الحالات الشاهدة مقابل الكثافة في داء السكري النوع ٢.

الاستنتاج: لم يكن لدى مرض السكري معدلات أعلى لحمولة المبيضات فحسب، بل وجدنا أصنافاً متنوعة من المبيضات مقاومة للمضادات الفطرية من فئة آزول. يترابط استعمار المبيضات لجوف الفم بصورة واضحة مع نسبة انضباط معدل سكر الدم ونوع السكري وحموضة اللعاب pH.

الخلاصات العربية

المدونات الطبية السعودية

تصدر المدونات الطبية السعودية مرة كل شهرين عن مستشفى الملك فيصل التخصصي ومركز الأبحاث ، ص.ب ٣٣٥٤ . الرياض ١١٢١١. المملكة العربية السعودية. يتم توزيع المدونات وإعداد الملخصات العربية بالتعاون مع د. زهير القاوي بواسطة موظفي إدارة الخدمات الإدارية بمستشفى الملك فيصل التخصّصي ومركز الأبحاث. جميع المراسلات والاستفسارات وطلبات الاشتراك ترسل إلى المحرر الأول مكتب المطبوعات (رإ ٣٦).

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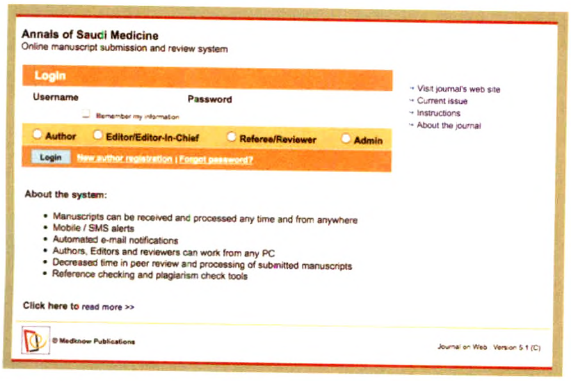

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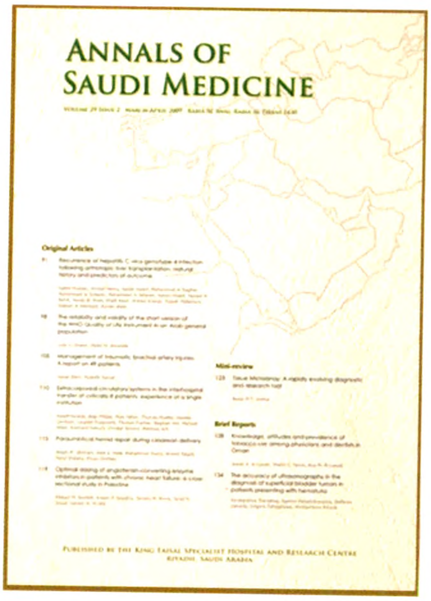

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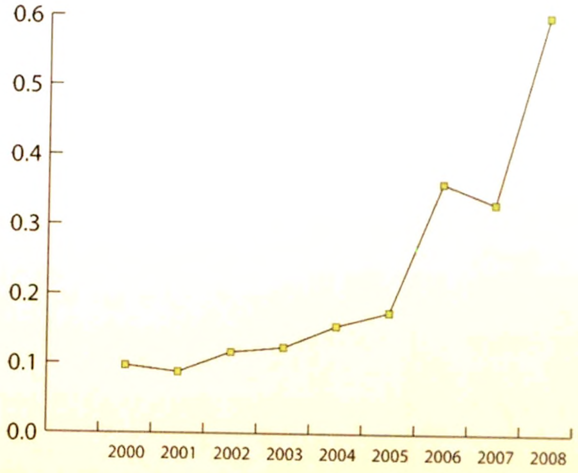

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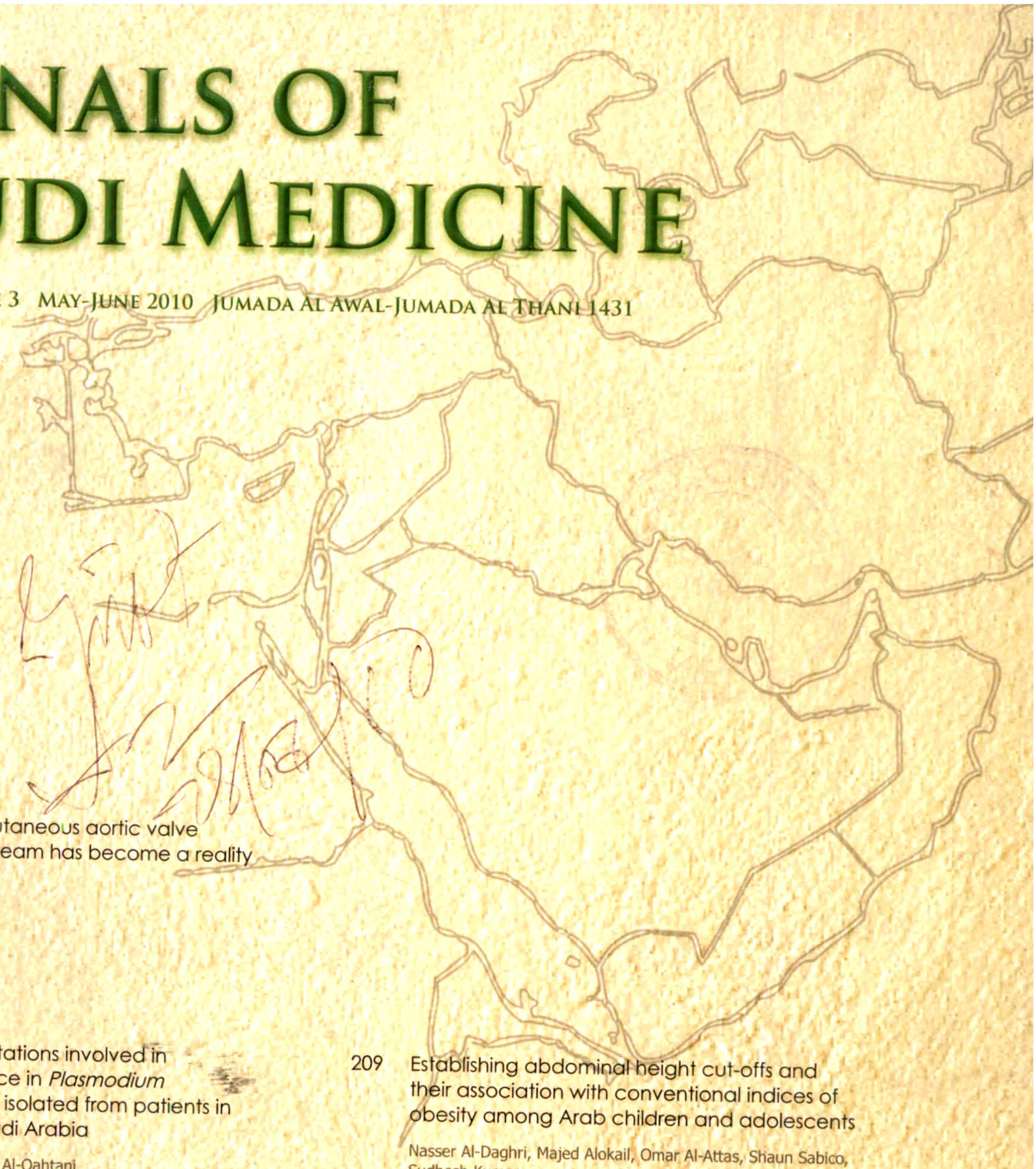

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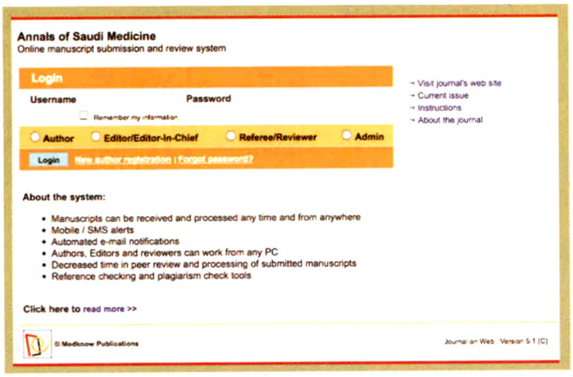

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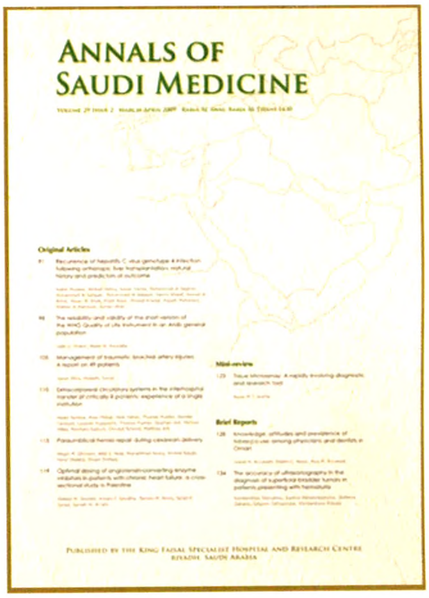

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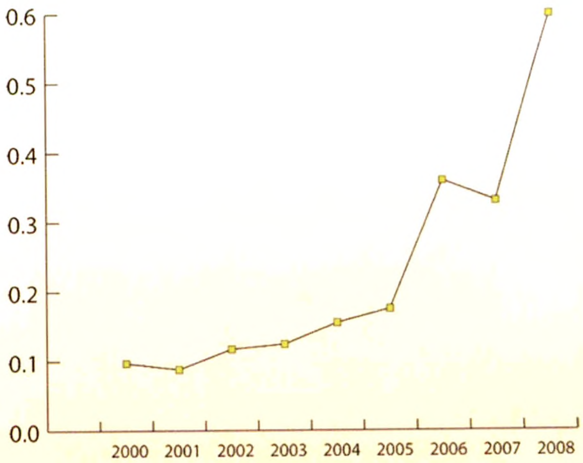

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09-10 March 2010 / 23-24 Rabi Al Awal 1431
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27-31 March 2010 / 11-15 Rabi Al Thani 1431
Contact No.: 4427754 (Surgery)

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APRIL (3)

1st Annual Pediatric Nephrology Meeting
06-07 April 2010 / 21-22 Rabi Al Thani 1431
Contact No.: 4427798 (Pediatrics)

Medication Safety: A Mission for All Symposium
13-14 April 2010 / 28-29 Rabi Al Thani 1431
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International Symposium on New Frontiers in Breast Cancer
27-29 April 2010 / 13-15 Jumada Al Awal 1431
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MAY (7)

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04-05 May 2010 / 20-21 Jumada Al Awal 1431
Contact No.: 4427224 (Pathology & Laboratory Medicine)

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05 May 2010 / 21 Jumada Al Awal 1431
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11-12 May 2010 / 27-28 Jumada Al Awal 1431
Contact No.: 4424988 (Medical Genetics)

12th Clinical Research Professionals' Course
15-26 May 2010 / 01-12 Jumada Al Thani 1431
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18-19 May 2010 / 04-05 Jumada Al Thani 1431
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All manuscripts reporting the results of experimental investigation involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after approval of the experimental protocol by a local human ethics committee. (Revised April 2010)

FROM THE EDITOR-IN-CHIEF

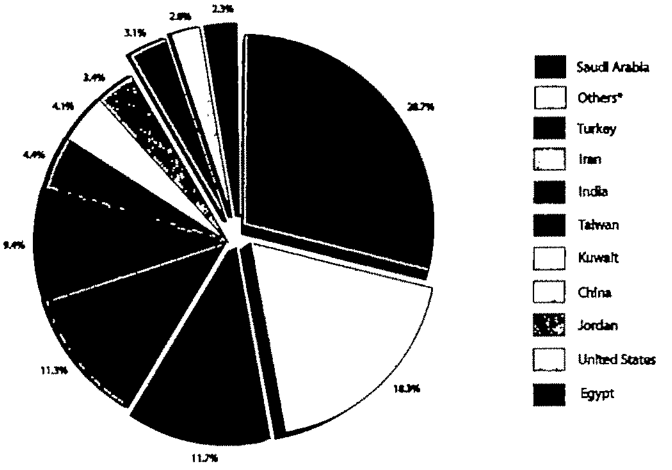
> *"The art of progress is to preserve order amidst change, and to preserve change amidst order,"*
> - Alfred North Whitehead, an English mathematician, logician and a philosopher

The baton of Editor-in-Chief was passed on to me in January 2010. To start out with, I would like to acknowledge the exemplary job done by my predecessors, the editorial board members and the editorial staff. In this regard, and within this short period of time I have come to recognize and have developed tremendous appreciation for a grand job by my immediate predecessor, Dr. Ali Zahrani. Indisputably, he has provided unparalleled leadership. The transition has been seamless. I have accepted the responsibility of the Editor-in Chief with joy and pleasure, coupled with an immense sense of responsibility.

The fact that the *Annals* now enjoys the highest impact factor in the region is a direct tribute to the efforts over the last few years of previous Editors-in-Chief, the eminent members of the editorial board and the editorial staff. Recently, in an unsolicited comment, Dr. Kenneth L. Mattox, one of the pioneering surgeons from the early days of KFSHRC, congratulated the editorial board on bringing "this high impact medical journal so very far" in documenting "the tremendous advancement you have made in providing advanced medical care to your citizens and visitors" (full comment to appear in the July issue).

By now the journal has grown to become one of the most important general medical journals, not only in the Middle East, but the Eastern Mediterranean and Asia, with manuscripts submitted from all over the world. The journal is included in the National Library of Medicine's MEDLINE database, searchable through PubMed.

Despite improvements of the last few years, there are still challenges that remain to be resolved. The wide popularity of the *Annals* has resulted in a record-breaking number of submissions of high quality articles averaging about 40 submissions per week. We have been receiving submissions from a wide and vast area as reflected in the accompanying figure. Coping with this demand is a pleasant yet demanding task.



*Countries under 2%: Morocco, Malaysia, Pakistan, Bahrain, Croatia, Iraq, UAE, Bosnia and Herzegovina, Qatar, Italy, Nepal, Nigeria, Australia, United Kingdom, Yemen, Greece, Canada, Mexico, Tunisia, Algeria, Bangladesh, Brazil, Chile, Ethiopia, Georgia, Ireland, Lebanon, New Zealand, Oman, Occupied Palestinian Territory, Poland, Serbia

Notwithstanding an excellent peer review and production, we need to maintain a mean submission to first decision time of 31 days, and speed up the time to first decision and time to publication. By necessity, I have expanded the membership to the Editorial Board. I am overjoyed that several talented and dedicated clinicians and scientists have accepted the challenge with enthusiasm and respect. Also, the Editorial Board is honored to add three international figures: Dr. Peter Hall, of Queen's University Belfast, UK, and Editor-in-Chief of The Journal of Pathology, Dr. Beverly Teischer, Senior Editor of Clinical Cancer Research, and Vice President of Oncology Research, Genzyme Corporation, from Massachusetts, USA, and Dr. Edward Laws, a celebrated neurosurgeon of Brigham and Women's Hospital, Boston, USA.

Other issues to contend with include: 1) Should there be a limitation on the number of authors? 2) How best to solve the shortage of reviewers who are qualified and willing to help the *Annals* make decisions, and 3) Reviewers need to be reminded to be constructive in criticism, thus allowing authors to revise their papers to a higher standard for resubmission.

A new item we have initiated is the "Editor's Corner," a series of mini-reviews that will reflect contemporary cutting-edge information. The review by Walid Hassan, MD, on transcatheter percutaneous aortic valve implantation is the first in the series.

Lastly, but not least, I would like to thank the authors, the reviewers, the Board members and the staff for their continued tireless efforts to have made the *Annals* the success it enjoys. Collectively, we have great powers to achieve formidable tasks. However, "Do not pray for tasks equal to your powers; pray for powers to be equal to your task." This was a realistic admonition offered by Phillips Brooks, an author from Massachusetts. Our task at the *Annals* is certainly a noble one.

Mohammed Ahmed MD, FACP, FACE, FRCP
Editor-in-Chief

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SUMMARIES

INFECTIOUS DISEASE

Analysis of gene mutations involved in chloroquine resistance in *Plasmodium falciparum* parasites isolated from patients in the southwest of Saudi Arabia

Al-Qahtani and Bin Dajem examined the molecular basis of chloroquine resistance among *Plasmodium falciparum* from the southwestern region of Saudi Arabia by studying two genes implicated in resistance to chloroquine. Mutations associated with chloroquine resistantance were found in most samples in this first study on the existence of the mutation in the PfMDR1 gene in the country.

Invasive pulmonary aspergillosis in acute exacerbation of chronic obstructive pulmonary disease and the diagnostic value of combined serological tests

Gao and colleagues found that the rate of proven and probable invasive pulmonary aspergillosis in patients with AECOPD was 1.91%. The combined galactomannan antigen and 1,3-β-glucan tests were useful for identifying false-positive reactions than either test used alone, but the value of this approach needs to be proven in future trials.

HEMATOLOGY

Recombinant activated factor VII in controlling bleeding in non-hemophiliac patients

In 45 non-hemophiliac patients identified by AlGahtani and colleagues, there was a marked and significant reduction in transfusion requirements for packed red blood cells and overall transfusion requirements significantly decreased after the infusion of rF-VIIa. The use of rFVIIa might be warranted in specific situations, but prospective, randomized studies are needed to investigate the efficacy and cost-effectiveness of rFVIIa for this indication.

FAMILY MEDICINE

Prevalence of overweight and obesity in Saudi children and adolescents

Using 2005 Saudi reference data on 19 317 healthy children and adolescents from 5 to 18 years of age, El Mouzan and colleagues found that the overall prevalence of overweight, obesity and severe obesity in all age groups was 23.1%, 9.3% and 2%, respectively. The report establishes baseline national prevalence rates, which are intermediate between developing and industrialized countries.

Establishing abdominal height cut-offs and their association with conventional indices of obesity among Arab children and adolescents

Al Daghri and colleagues document for the first time the association between sagittal abdominal diameter or abdominal height and conventional markers of obesity in 964 Arab children and adolescents. They found that it was a reliable indicator of visceral obesity, but prospective studies are needed to determine whether abdominal height is risk factor for hard endpoints such as diabetes mellitus and coronary heart disease.

editor's corner

Transcatheter percutaneous aortic valve implantation: The dream has become a reality

Walid M. Hassan

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Aortic valve stenosis (AS) is increasing in frequency as the population ages.[1] Severe AS is common, and when symptomatic, is associated with a predictably poor prognosis and high mortality.[2,3] Although surgical aortic valve replacement (AVR) is still the gold standard for symptomatic patients with severe AS,[4,5] in a sizable subset of high-risk patients the surgical option is excluded due to severe comorbidities and advanced age. In addition, several registries show that referring physicians often do not propose surgery; 33% of patients with severe symptomatic AS in the Euro Heart Survey were not being considered for surgery.[6] Thus, despite the good results of valve surgery, there may well be a role for less invasive alternatives.

Why Percutaneous Valve Implantation?

Balloon aortic valvuloplasty has a limited role in providing temporary relief of symptoms with high recurrence rates and a 1-year survival rate of only 54%.[7] Its main role is palliation or as a bridge to surgical AVR. Medical therapy for these patients is associated with a dismal outcome.[8] Thus, transcatheter percutaneous aortic valve implantation (TAVI) has recently emerged as an alternative to surgical AVR. This was first demonstrated in an animal model by Andersen et al in 1992, who delivered a porcine bioprosthesis attached to a wire-based stent at various aortic sites with satisfactory hemodynamic results.[9]

Since the first TAVI in a human in 2002,[10] percutaneous heart valves (PHV) have already undergone several modifications from the first-generation devices. Currently, two PHV devices have been CE-marked (Conformité Européenne) and are in clinical application: a balloon-expandable and a self-expandable PHV. Despite initial skepticism, TAVI is becoming a clinical reality, with over 6000 procedures performed to date in many countries. Initial experience suggests that outcomes compare favorably with conventional valve surgery in selected patients. However, in the future a more mature procedure might represent a viable alternative for a much broader range of patients.

Current Status

Currently, there are two PHVs in clinical trials, the balloon-expandable Cribier-Edwards (recently, the Edwards-Sapien) valve (Edwards Lifesciences Inc., Irvine, California), and the self-expandable CoreValve (CoreValve, Irvine, California). The balloon-expandable PHV consists of three pericardial leaflets, initially equine (Cribier-Edwards) and currently bovine (Edwards-Sapien), mounted within a tubular, slotted,

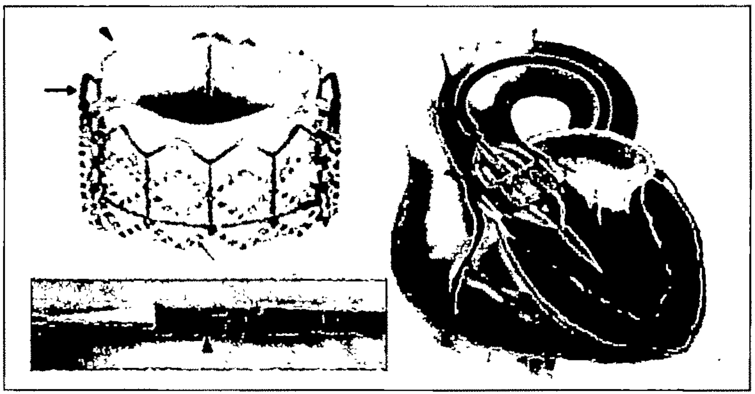

Figure 1. Edwards-Sapien percutaneous heart valves. Bovine pericardium leaflets (blue arrowhead) sutured (expanded polytetrafluoroethylene) (black arrow) onto a stainless steel stent frame (blue arrow) (Top). Delivery catheter with valve loaded (black arrowhead) (Bottom).

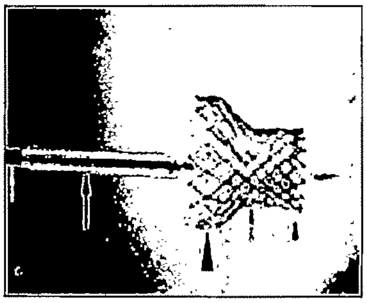

Figure 2. CoreValve PHV. A 12-F delivery catheter shaft (white arrow), 18-F delivery case, which contains valve (black arrow) with a flexible distal nose cone; opened valve consisting of the lower (inlet) portion (small arrowhead), the constrained middle portion to allow coronary perfusion (red arrow), and the upper (outlet) portion (large arrowhead).

stainless steel balloon-expandable stent (Figure 1). Current generation devices require either a 22-F or 24-F sheath for delivery.[11] This PHV was initially implanted via the antegrade transseptal approach. There were several problems with this approach, and the retrograde approach has since been shown to be safer with the use of a proprietary steerable delivery catheter.

The self-expandable PHV (CoreValve) consists of three pericardial tissue leaflets, initially bovine and currently porcine, mounted and sutured in a self-expandable nitinol stent (Figure 2). The stent frame is 50 mm, with the lower (inlet) portion having a high radial force to expand and exclude the calcified aortic leaflets; the middle portion carries the valve. The coaptation point of the leaflets is actually supra-annular- and is constrained to avoid obstructing the coronary arteries. The upper portion (outlet) is flared to fixate the stent in the ascending aorta and provide longitudinal stability. Early generation devices required 25-F sheaths; later devices incorporated porcine pericardial tissue constrained within 21-F, and now 18-F sheaths.[12] Other valves and delivery systems with potential advantages in terms of the ability to deliver, deploy, or reposition the prosthesis are under development and in early pre-clinical evaluation.

Indications

To date TAVI has been performed only in patients at high surgical risk. Objective estimates of surgical mortality have been used to help define "high-risk" like a logistic EuroSCORE >20 or Society of Thoracic Surgeons (STS) score >10. The other set of patients who may be considered at present are those with a deteriorated aortic bioprosthesis deemed at high risk for surgical reoperation, and this "valve-in-valve" concept has already been reported. With technological advancements, it is expected that the ease of implantation will improve and complications will decrease.

Contraindications

The major specific contraindication to TAVI via the femoral artery is the presence of severe ilio-femoral stenosis. Some patients might have an aortic annulus that is too small or too large (current prostheses are suitable for an echocardiographic annulus diameter between 18 and 26 mm). Others might have an unusually bulky valve at risk of obstructing a coronary ostium. Mitral insufficiency and non-revascularized coronary disease are not necessarily contraindications; both are often well-tolerated in elderly patients, once aortic stenosis is relieved. However severe left ventricular dysfunction, severe mitral valve disease or nonrevascularized coronary disease can predispose to hemodynamic instability during TAVI.

Procedure

The TAVI is typically performed in a cardiac catheterization laboratory or hybrid operating room. General anesthesia is optional. Femoral artery access and hemostasis might require a cutdown, but is increasingly accomplished with percutaneous closure. Conventional balloon valvuloplasty is initially performed. The valved stent is typically introduced into a femoral sheath and passed through the aorta. Positioning of the prosthesis within the native valve is confirmed with fluoroscopy, aortography, and often transesophageal echocardiography. Burst pacing might be used to transiently reduce transvalvular flow during deployment of balloon-expandable valves. The valved stent is expanded within the native aortic valve, displacing and excluding the diseased leaflets and substituting a new functional valve in place of the old. Discharge occurs as early as day 2, although median discharge might average 5 days as a consequence of delayed mobilization and disposition of elderly patients.

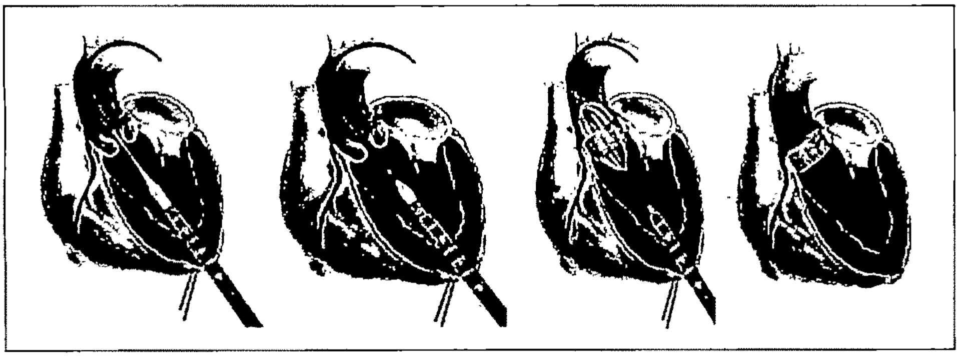

Figure 3. Transapical transcatheter percutaneous aortic valve implantation.

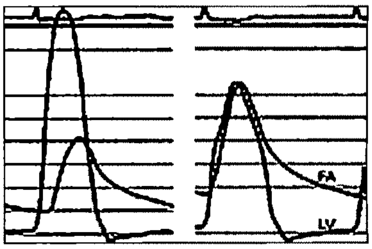

Figure 4. Transaortic gradients. Left ventricular (LV) to aortic gradients before and after percutaneous aortic valve replacement. FA=femoral artery.

Transapical Procedure

An alternative route of percutaneous valve implantation is via the open chest equivalent, which allows extension of the "transcatheter" technology to patients with vascular disease. This approach uses a mini-thoracotomy and needle puncture of the apical left ventricle without cardiopulmonary bypass (Figure 3). Reported experience is favorable, and as of early 2010 approximately 2000 transapical valve implantations have been performed worldwide.

Complications

Of great concern is the possibility of arterial dissection or perforation while manipulating very large catheters through the vasculature. Myocardial ischemia or cardiogenic shock might occur and of concern is coronary obstruction by the prosthetic or native valve, although this seems infrequent. As with aortic valve surgery, TAVI is associated with a finite risk of atrioventricular block and pacemaker dependence. Reported rates of stroke with transcatheter valve implantation vary from 3% to as high as 9%.[13] Hopefully these risks will diminish as the procedure improves and is applied to patients with less comorbidity.

Outcomes

Initial transarterial experience in "high-risk" or "inoperable" patients was favorable, with successful implantation increasing from 76% to 96% with experience. Intraprocedural mortality was low at 2%. Mortality at 30 days after the procedure was 12%, comparing favorably to a logistic EuroSCORE estimate of 28% in this high-risk group.[11]

Overall, procedural success is closely linked to experience and is about 90% in experienced centers. A learning curve can also be observed resulting in better patient selection and outcomes. Valve function is good with a final valve area ranging from 1.5 to 1.8 cm^2.

Mortality at 30 days ranges from 5% to 18%. Acute myocardial infarction occurs in 2% to 11%. Coronary obstruction is rare (<1%). Mild-to-moderate aortic regurgitation, mostly paravalvular, is observed in about 50% of cases. However, the availability of larger prostheses and their more careful matching with the size of the aortic annulus led to a decrease in the incidence of severe aortic regurgitation to about 5%. Prosthesis embolization is rare, about 1%. Vascular complications, with an incidence ranging from 10% to 15%, remain a significant cause of mortality and morbidity. Stroke ranges from 3% to 9%. Finally, atrioventricular block occurs in 4% to 8%, necessitating pacemaker implantation in up to 24% with self-expandable devices.

Long-term results up to 3 years (though only 1 year in most studies) are reported in a limited number of patients. They show a survival rate of 70% to 80% with a significant improvement in clinical condition in most cases. Serial echocardiographic studies have consistently shown good prosthetic valve function with no structural deterioration of valve tissue.

Valve Function

In vitro and clinical evaluations of currently available transcatheter valves demonstrate excellent valve function (Figure 4). Orifice areas are typically larger than comparable surgical prostheses, owing to the absence of a bulky sewing ring and the ability to implant oversized prostheses after balloon dilation. Echocardiographic evaluation of both currently available valves typically documents gradients of fewer than 10 mm Hg and effective orifice areas of over 1.5 cm^2. Although paravalvular regurgitation remains ubiquitous, such leaks are generally mild and hemolysis has not been observed.

Late Outcomes

Acute and sustained improvements in left ventricular systolic function, functional mitral insufficiency, and functional class have been demonstrated after TAVI. In the high-risk candidates currently undergoing these procedures, late survival seems limited by comorbidities rather than valvular or coronary disease. Currently, on-going randomized controlled trials (PARTNER US and PARTNER EU) will help to determine the future of this revolutionary and promising interventional procedure. Physicians at King Faisal Heart Institute were among the first in the region to implement this sophisticated program with a very encouraging early result.

Conclusions

The currently available results obtained with TAVI suggest that these techniques are feasible and provide hemodynamic and clinical improvement for up to 3 years in patients with severe symptomatic AS at high risk or with contraindications for surgery. Pending questions concern mainly safety and long-term durability. Surgeons and cardiologists must work as a team to select the best candidates, perform the procedure, and, finally, evaluate the results. Today, these techniques are targeted at high-risk patients but they may be extended to the lower risk groups in the future, if the initial promise holds true after careful evaluation. The road is long and arduous, but the dream has become a reality.

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original article

Analysis of gene mutations involved in chloroquine resistance in *Plasmodium falciparum* parasites isolated from patients in the southwest of Saudi Arabia

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BACKGROUND AND OBJECTIVES: Chloroquine has been the drug of choice for the treatment of malaria for many decades. We aimed to examine the molecular basis of chloroquine resistance among *Plasmodium falciparum* isolates from the southwestern region of Saudi Arabia by analyzing the K76T and N86Y mutations in the PfCRT and PfMDR1 genes, respectively.

PATIENTS AND METHODS: *P falciparum*-infected blood spot samples (n=121) were collected on filter papers. DNA was extracted and fragments from the above genes were amplified using nested PCR. The amplicons were digested by ApoI enzyme and sequenced.

RESULTS: Of the 121 samples, 95 and 112 samples could be amplified for PfCRT K76T and PfMDR1 N86Y mutations, respectively. All of the samples amplified for the PfCRT K76T mutation were undigestible by ApoI, suggesting the presence of the K76T mutation. For the PfMDR1 N86Y mutation, 65/109 samples (59.6%) were digestible when treated with ApoI in a pattern, suggestive of the presence of the investigated wild allele (N86). However, 44/109 samples (40.4%) were digestible by ApoI, suggesting the presence of the mutated allele (Y) at position 86. DNA sequencing confirmed these results.

CONCLUSION: Surprisingly, all isolates exhibited the mutated allele at codon 76 (K76T) of PfCRT. However, the mutated mutant allele at codon 86 (N86Y) of PfMDR1 was found in 40.4% of the samples studied. To our knowledge, this is the first study that has investigated the existence of the mutation in the PfMDR1 gene in the country. This study will contribute to the development of new strategies for therapeutic intervention against malaria in Saudi Arabia.

Malaria is a serious global public health problem caused by the protozoan parasites belonging to the genus *Plasmodium*. Whereas *Plasmodium vivax*, *P malariae*, and *P ovale* give rise to considerable malaria morbidity, only *P falciparum* results in high mortality. The disease is considered to be re-emerging, largely due to the spread of malarial drug resistance, insecticide resistance, and the rise in population and travel.[1] About 40% of the world's population lives in areas of malaria transmission in nearly 100 countries, and infection with malaria results in 1.1-2.7 million deaths annually.[2,3]

According to the World Health Organization Eastern Mediterranean Region (WHO-EMR) report, Saudi Arabia is considered an endemic country for malaria transmission,[4] with most cases diagnosed in the Jazan and Aseer regions located in the southwest part of the country. Nearly 96% of the cases are caused by *P falciparum* with *Anopheles arabiensis* being the main vector for transmission.[5-8]

The malaria control program in Saudi Arabia is based mainly on integrated control measures, including indoor spraying of insecticides, case detection, and treatment. High rates of population movement of expatriates working in the country and pilgrims between Saudi Arabia and malaria-endemic countries, such as

Sudan, India, and Yemen, complicate the control of the disease.[9,10]

Chloroquine is the drug of choice for the treatment of complicated malaria cases. However, another combination of two drugs (sulfadoxine and pyrimethamine [SP]) is also effective against the disease. The current mainstream view of chloroquine antimalarial action is that chloroquine kills malaria parasites by binding to its target, a nonprotein molecule, ferriprotoporphyrin IX in lysosomes (food vacuoles) to form a toxic complex that lyses the parasites.[11] The emergence and spread of chloroquine resistance (CQR) has been a problem for world health.[12] In Saudi Arabia, the strategy to treat malaria cases was changed in 2008 by the Saudi health authorities with the introduction of artemisinin combination therapy (ACT) that consists of SP and artemisinin.[13] Saudi Arabia is one of the few areas where *P falciparum* remained sensitive to chloroquine until the early 1990s despite the fact that CQR emerged in malaria-endemic countries close to Saudi Arabia, such as Iran, Pakistan, and East Africa where CQR was first reported in the early 1980s.[14,15] A recent study examined a limited number of samples from the Jazan region for *P falciparum* chloroquine-resistant transporter (PfCRT) gene and found a CQR rate of 89.5%.[16]

Over the years, the emergence of drug-resistant parasites has hampered the efforts to control malaria worldwide. Since the late 1950s, reports of CQR have been documented in all endemic areas. Mutations in two genes, namely, PfCRT and *P falciparum* multidrug-resistant gene 1 (PfMDR1) have been implicated in resistance to chloroquine.[17] Several point mutations in the PfCRT gene have been shown to correlate with resistance. Of these, only the mutation of lysine to threonine at the 76th position (K76T) in CRT is significantly found in resistant strains of malaria from different endemic areas of the world.[18,19] Other mutations observed in the PfMDR1 gene were found to be strongly linked to CQR in various regions.[20] Several field studies have related CQR to the mutation of aspargine to tyrosine at the 86th position (N86Y) of the MDR1 protein.[21,22] In this study, we examined the presence of K76T and N86Y mutations in PfCRT and PfMDR1, respectively, among *P falciparum* parasites isolated from the southwestern region of Saudi Arabia.

PATIENTS AND METHODS

The cases studied in this report were individuals (n=121) suspected of malaria infection who visit the Malaria Center in Aseer and Jazan Provinces. Only malaria cases confirmed with blood microscopic examination were included in this study. Blood samples (nearly 50 μL) were collected by finger-pricking and three drops from each patient were blotted onto Whatman (3 mm) chromatography paper. The spotted blood samples were placed in plastic bags and transported to the laboratory. All patients who participated in this study signed a consent form and the study was approved by the Ethics Committee at King Khalid University, Abha, Saudi Arabia.

DNA was extracted from the spotted blood samples according to the method of Sakihama et al with some modifications.[23] Briefly, filter papers containing blood spots were placed into 1.5 mL Eppendorf tubes and 0.5% saponin was added and incubated overnight at 4°C. One hundred microliters of 5% Chelex-100 solution (Bio-Rad Laboratories, USA) were added to each tube and incubated at 100°C for 5 minutes in a heating

Table 1. Primers, expected molecular weight of amplicons, and the PCR conditions for genes investigated in this study.

Primers		Sequence (5'–3')	Size (bp)	PCR conditions
First round				
PfMDR1	OMDR1/F	TGTTGAAAGATGGGTAAAGAGCAGAAAGAG	660	94°C, 3 min followed by 45 cycles (94°C, 30 sesc; 56 °C, 30 secs; 60°C, 30 secs); 60°C, 3 min
	OMDR1/R	TACTTTCTTATTACATATGACACCACAAAC		
PfCRT	OCRT/F	CCGTTAATAATAAATACACGCAC	1389	
	OCRT/R	CGGATGTTACAAAACTATAGTTAC		
Second round				
PfMDR1	IMDR1/F	GTCAAACGTGCATTTTTTATTAATGACCATTTA	560	94°C, 3 min followed by 40 cycles (94°C, 30 secs; 47°C, 30 secs; 68°C, 1 min); 64°C, 3 mins
	IMDR1/R	AAAGATGGTAACCTCAGTATCAAAGAGAG		
PfCRT	ICRT/F	TGTGCTCATGTGTTTAAACTT	145	
	ICRT/R	CAAAACTATAGTTACCAATTTTG		

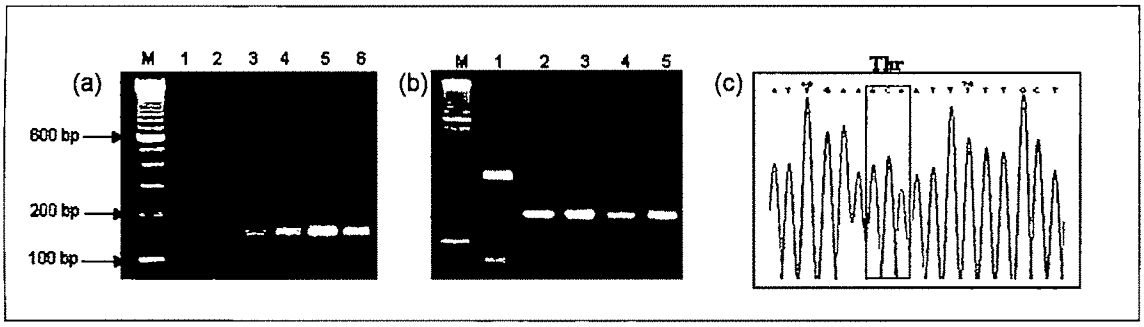

Figure 1. (a) Agarose gel of typical PCR amplification patterns of the nested PCR products of the DNA from *P falciparum*-infected blood samples for PFCRT gene, showing the expected product of 154 bp. M: 100-bp ladder, lanes 1 and 2: no-template negative controls, lanes 3-6: PCR product from representative patient samples. (b) Patterns obtained after restriction digestion of the PCR products with ApoI. Lane 1: restriction digestion control, lanes 2-5: PCR product from representative patient samples after digestion with ApoI. (c) Chromatogram confirming the presence of mutation at the 76th amino acid position highlighted in yellow.

block with vortexing every one minute. The supernatant was transferred to a new tube and stored at 4°C until further processing.

The primers used in this study were originally based on published data.[24] A set of outer and inner primers was synthesized for the amplification of DNA fragments from PfCRT and PfMDR1 genes. Primer sequences, expected molecular weight of amplicons, and the PCR conditions are illustrated in Table 1. For digestions, aliquots of PfCRT and PfMDR1 PCR products were digested with 10 U of the restriction enzyme ApoI (New England Laboratories, USA), according to the manufacturer's recommendations. All digested products were electrophoretically processed in 2% agarose gels and the bands were visualized with 1 μg/mL ethidium bromide under UV light. DNA sequencing was carried out at the DNA sequencing core facility at the Research Center of King Faisal Specialist Hospital and Research Center (KFSHRC), using BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's instructions (BigDyeTerminator v3.1 Cycle Sequencing Kit, Applera, USA) and the sequences were analyzed using DNASTAR software.

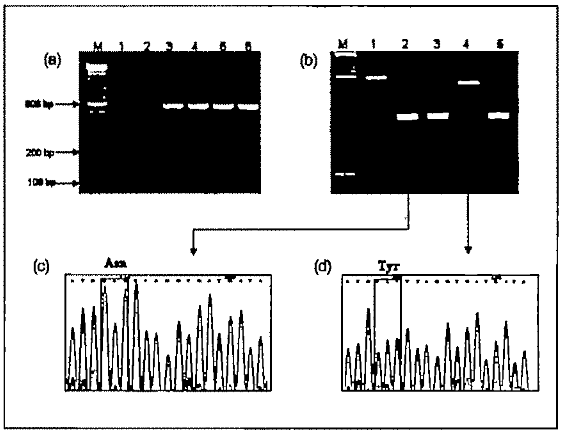

Figure 2. (a) Agarose gel of typical PCR amplification patterns of the nested PCR products of the DNA from *P falciparum*-infected blood samples for PFMDR1 gene, giving the expected product of 560 bp. M: 100-bp ladder, lanes 1 and 2: no-template negative controls, lanes 3-6: PCR product from representative patient samples. (b) Patterns obtained after restriction digestion of the PCR products with ApoI. The products of the wild-type allele are 250 bp, 231 bp and 79 bp, while, two fragments of 481 bp and 97 bp are evident for the mutant alleles. M: 100-bp ladder, lanes 1: undigested PCR product, lanes 2-5: PCR product from representative patient samples after digestion with ApoI. (c and d) representative chromatograms confirming the presence of mutant and wild alleles at the 86th amino acid position.

RESULTS

Analysis of the PfCRT K76T codon

Of the 121 samples examined, 26 (21.5%) were not amplifiable but the remaining 95 samples (78.5%) gave amplification products (Table 2) and the amplicons of expected molecular weight (145 bp) were visualized after nested PCR (Figure 1a). None of the 95 amplified samples (100%) could be digested by ApoI, suggesting the presence of the mutation of interest (76T) that indicates chloroquine resistance (Figure 1b). DNA sequencing of the PCR products of these samples confirmed the results obtained by the PCR/RFLP method (Figure 1c).

Analysis of the PfMDR1 N86Y codon

Of the 121 samples analyzed, 112 samples (92.5%) yielded amplification products (Table 2) and the amplicons of expected molecular weight (560 bp) were visualized after nested PCR (Figure 2a). Of the total

Table 2. Prevalence of wild and mutant alleles of PfCRT and PfMDR1 conferring resistance to chloroquine in *P falciparum* isolates from Southwest parts of Saudi Arabia.

Gene	N	Wild Allele	Mutant Allele	Mutation (%)
PfCRT	95	76K (0/95)	76T (95/95)	100
PfMDR1	109	86N (65/109)	86Y (44/109)	40.4

samples examined, 9 (7.5%) were not amplifiable under the conditions used in this study. This suggested that these samples could have been misdiagnosed for malaria or that it could have been a form of malaria caused by a species other than *P falciparum*. Of the 109 correctly amplified samples, 65 (59.6%) gave 250, 231, and 79 bp bands after ApoI digestion, indicating the presence of the wild-type 86N codon. On the other hand, 44 (40.4%) samples gave 481 and 97 bp bands after ApoI digestion, indicating the presence of the mutated 86Y codon (Figure 2b). The remaining three (of the 112 yielding amplification products) samples showed faint bands that were not appropriate for the digestion assay. DNA sequencing confirmed the results obtained by PCR/RFLP analysis (Figure 2c and 2d).

DISCUSSION

At present, there is little information on the association of genetic variation in *P falciparum* parasites with drug resistance in Saudi Arabia. As many field and laboratory studies have investigated the association of CQR with specific mutations in codon 76 of the PfCRT gene and at codon 86 of the PfMDR1 gene, we aimed to investigate these mutations by PCR-RFLP and DNA sequencing technologies in isolates from the southwestern part of the country. Chloroquine was introduced in the 1940s as the drug of choice for malaria treatment.[25] However, parasite resistance to chloroquine appeared after more than 60 years of intensive use. Extended parasite exposure to chloroquine has necessitated the exploration of the characteristics of *P falciparum* population genetics in relation to resistance to chloroquine.

We used standard molecular biology methods to study mutations linked to resistance in PfCRT and PfMDR genes.[24] However, not all samples were amplifiable as only 95 and 112 samples were amplified for the PfCRT K76T and PfMDR1 N86Y mutations, respectively. Such problems have been documented with different PCR conditions. For example, Sutherland et al were able to amplify PfCRT in 98/108 (90.7%) samples and to amplify PfMDR1 in 74/108 samples (68.5%).[26]

When the PfCRT gene was identified, many studies confirmed the presence of the K76T mutation in CQR parasites.[27] The investigation of another gene, PfMDR1,[28] revealed that a mutation at the 86th position was strongly linked to CQR in laboratory clones, even though it is not the sole requirement for CQR.[29] Globally, it has been found that replacement of the lysine (a positively charged amino acid) in PfCRT with threonine (an uncharged amino acid) at position 76 confers resistance to chloroquine.[30] The very high prevalence of the PfCRT 76T variant in our study is in agreement with the findings of other studies where clinical chloroquine treatment failure was evident.[31] A study from Iran reported that PfCRT 76T was found in 99% of the investigated samples.[32] In a recent prevalence study reported from Thailand, the PfCRT 76T allele frequency was found in 99.1% of the investigated samples.[33] A molecular prevalence survey has shown that the prevalence of the PfCRT 76T mutation was over 90% in Yunnan province, China.[34] A study of PfCRT point mutations and the level of CQR in *P falciparum* isolates imported into Europe from Congo and Tanzania showed that the frequency of the 76T mutated allele was 71.4%.[35] The presence of the PfCRT K76T mutation from Papua New Guinea was found to be 92.9%.[36] A recent study from the Philippines describing *P falciparum* isolates from three areas of the country showed that the frequency of the PfCRT 76T mutation was found to be 100% in Kalinga, 80% in Palawan, and 87% in Mindanao.[37]

Different PfCRT haplotypes have been reported from malarial isolates in endemic areas. The CQR-associated haplotype (amino acids from 72 to 76) detected in this study was the CVIET. This is considered the typical haplotype isolated from Southeast Asia and the African continent.[38] Finding the same haplotypes in Saudi Arabia could be attributed to the continuous travel and human migration related to employment, tourism, or religious pilgrimage.[39,40]

Currently, mutations detected in PfMDR1 have been hypothesized to augment the level of resistance in CQR *P falciparum* parasites.[24,41] In our study, the PfMDR1 86Y allele frequencies were found to be 59.6% and 40.4% for the N86 allele. A similar finding was reported from Iran where it was found that PfMDR1 86Y appeared in 72% of the samples in the Sistan-Baluchistan Province.[31] In Thailand, the PfMDR1 86N and 86Y alleles were identified in 75.5% and 20% of samples, respectively.[32] Isolates from three distinct areas of the Philippines showed that the frequency of the PfMDR1 N86Y mutation was 39% in Kalinga town, 35% in Palawan town, and 93% in Mindanao town isolates.[36] The PfMDR1 N86Y mutation was found in 23.1% of the isolates from Southeast Iran,[42] which is a frequency pattern similar to that found in African regions where CQR is well established.[43] Babiker and colleagues reported that the PfMDR1 N86Y mutation contributes more to CQR in the PfCRT 76T background in Sudanese isolates of *P falciparum*.[44] Our PCR-RFLP analysis consistently showed incomplete digestion of the PCR product, which is suggestive of multiclonal infection of the same parasites.[45] The molecular markers for CQR, when optimized and validated as tools for surveillance, will help in malaria control, guide national malaria treatment policies, and monitor changes in parasite drug susceptibility following changes in malaria drug treatment policy.

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original article

Invasive pulmonary aspergillosis in acute exacerbation of chronic obstructive pulmonary disease and the diagnostic value of combined serological tests

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BACKGROUND AND OBJECTIVES: Invasive pulmonary aspergillosis (IPA) among patients with chronic obstructive pulmonary disease (COPD) is increasing in frequency. We conducted this study to find out the approximate incidence of IPA in patients with acute exacerbation of COPD (AECOPD), and to determine whether using a combination of two tests (galactomannan [GM] antigen and 1,3-β-glucan [BG] detection) would result in a more specific diagnosis of IPA.

METHODS: The study included 261 patients with AECOPD admitted over two years. The patients were categorized according to the modified definitions for IPA. GM antigen and BG were detected by the Platelia *Aspergillus* and Glucatell tests.

RESULTS: Two patients had proven IPA, three had probable IPA, and two had possible IPA. The rate of proven or probable IPA in patients with AECOPD was 1.91% (5/261). Four patients with proven and probable IPA had been treated with a systemic or inhaled corticosteroid before hospitalization and the typical symptoms and diagnostic signs of IPA were relatively less common in them. Mortality in patients with proven and probable IPA was 80%. The specificity of combined GM and BG detection was 98.8%.

CONCLUSION: Combining two serological tests increased the specificity of diagnosis but further trials are needed to prove the value of this approach.

Over the last decade, the incidence of invasive aspergillosis (IA) has significantly increased in neutropenic patients and matched unrelated donor transplant recipients.[1] This severe opportunistic fungal infection is characterized by a high mortality rate in these at-risk patients.[2] Several publications indicate that the population at risk for pulmonary or disseminated IA should be expanded to include patients with chronic obstructive pulmonary disease (COPD).[3,4]

A systematic review showed that among 1941 patients with IA, 26 patients (1.3%) had COPD.[5] In one large study, 9% of 595 patients with IA suffered from some form of pulmonary disease.[6] Rodrigues et al reported that COPD patients constituted 1% of all cases of IA at their institution.[7] Recently, Guinea et al reported that invasive pulmonary aspergillosis (IPA) affected at least 22.1% of patients with COPD and that *Aspergillus* could be isolated by culture.[8] Although these numbers are impressive, the true incidence of IPA in COPD is probably underestimated, especially in a high-risk subset of patients, for example, those with acute exacerbation of COPD (AECOPD).

Establishing a diagnosis of IPA remains a challenge. Cultures may require days or weeks to grow, while the invasive procedures needed to obtain specimens for histopathological examination can rarely be performed on many patients. In the past decade, noninvasive diagnostic tests have focused on the detection of surrogate markers such as the galactomannan (GM) antigen and 1,3-β-glucan (BG). One study showed that the determination of GM in the sera of non-neutropenic patients could prove useful for diagnosing IA.[9] However, the use of these new diagnostic tools is hampered by a high rate of false-positive results.[10] The aim of this study was to find the approximate incidence of IPA in patients with AECOPD and whether a combination of two tests-GM and BG detection-would result in a more specific diagnosis of IPA.

METHODS

We performed this prospective study from October 2006 to November 2008 at No. 150 Hospital of the Chinese People's liberation Army (PLA), China. There are 1100 beds in this hospital, with 60 beds in the respiratory diseases department. All patients diagnosed with AECOPD during the study period were included. Patients with AECOPD were classified according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[11] The following data were collected for each patient: age, gender, accompanying disease, details of steroid treatment and broad-spectrum antibiotic received, history of mechanical ventilation, peripheral white blood cell count, radiographic findings, and frequency of hospitalization during the previous year, hospitalization duration, and outcome. Respiratory specimens were cultured on conventional medium, and selected specimens were further cultured on fungal media for at least 7 days. *Aspergillus* was identified by standard morphological procedures. A blood sample of each patient was obtained after the preliminary diagnosis had been made. Serum samples were stored at −20°C until use. This study was performed with the approval of the Ethics Committee of the Chinese People's Liberation Army No. 150 Hospital. Informed consent was obtained from all study subjects.

The EORTC/MSG (European Organization for the Treatment of Cancer/Mycoses Study Group) guidelines for IPA were not designed for patient categories other than cancer patients and bone marrow transplant recipients.[1] One important at-risk group, patients with COPD, was not included in this definition. So we used modified IPA definitions to classify our patients as follows:[1,3,4,12-14]

- 'Proven IPA' referred to those who had histopathologic or cytopathological evidence of tissue invasion (in needle aspiration or biopsy and/or autopsy specimen), with septated, acutely branching hyaline hyphae compatible with *Aspergillus* seen on microscopy, or culture of a sample from a normally sterile but clinically infected body site (excluding bronchoalveolar lavage fluid and sinus aspirate) obtained by a sterile procedure was positive for *Aspergillus* species.
- 'Probable IPA' referred to those with positive culture results or cytological evidence for *Aspergillus* species from a lower respiratory tract (LRT) specimen, in conjunction with one major sign (halo sign or 'air crescent' sign on CT scan) or at least two minor signs (LRT infection, pleural rub, and presence of any new infiltrate in a patient who did not fulfill the major criterion but for whom no alternative diagnosis was available). 'Probable IPA' was also applied to the COPD patient, usually treated with steroids, that had severe disease (stage III or IV) according to GOLD criteria, with recent exacerbation of dyspnea resistant to appropriate treatment (including antibiotics) and with suggestive chest imaging (pulmonary lesions shown by radiograph or CT scan to be unresponsive to appropriate antibiotics), accompanied by one of the following: i) Positive culture and/or microscopy for *Aspergillus* from LRT, ii) positive serum antibody test for *Aspergillus* fumigatus, iii) two consecutive positive serum GM tests.
- 'Possible IPA' referred to the COPD patient, usually treated with steroids, that had severe disease according to GOLD criteria (stage III or IV), with recent exacerbation of dyspnea that was resistant to appropriate treatment (including antibiotics) and with suggestive chest imaging, but without positive *Aspergillus* culture or microscopy from LRT or serology.
- 'Colonization' referred to those with positive culture for *Aspergillus* from a nonsterile site, but without any other evidence of fungal infection.

The detection of GM antigen by the Platelia *Aspergillus* EIA test (Bio-Rad Laboratories, Marnes, France) was carried out according to the manufacturer's instructions. A sample was considered positive if the index was ≥1.5.[15] BG was detected with the Glucatell test kit (Associates of Cape Cod, Falmouth, Massachusetts, US). Patients were judged positive if the level of BG was ≥80 pg/mL.[16] Testing for GM antigen and BG was done in batches. If the patient was still in hospital when the serology result was reported, the treating physician clinician received the result.

Sensitivity, specificity, and positive and negative predictive values were calculated as described by Kozinn.[17] According to Mennink-Kersten et al, only proven and probable IPA were considered truly positive and only no IPA cases were considered truly negative. Patients classified as possible IPA were not included in the analyses because of diagnostic uncertainty.[18]

RESULTS

The study included 261 patients with AECOPD who were admitted during the study period (October 2006 to November 2008). The mean (SD) age of patients was 65.3 (9.6) years. According to the GOLD severity classification, 105 (40.2%) patients were in stage I and II and 156 (59.8%) in stage III and IV. Seventy-one (27.2%) patients had a history of treatment with systemic or inhaled corticosteroid in the 3 months preceding admission, and 87 (33.3%) patients had been admitted on at least one occasion during the previous year. Among these patients, 2 had proven IPA, 3 had probable IPA, and 254 patients did not have IPA; the remaining 2 patients had possible IPA.

For the five IPA patients (proven and probable IPA), the main clinical sign was nonspecific antibiotic-resistant pneumonia associated with exacerbation of dyspnea. On hospital admission, four IPA patients had been treated with corticosteroids, and three received an accumulated dose (or equivalent) of prednisone of >700 mg over the last 3 months. All IPA patients were administered systemic corticosteroids during hospitalization, and four received a prednisone equivalent of >0.3 mg/kg/day. Among non-IPA patients (possible and no IPA), 28 (10.9%) and 39 (15.2%) patients, respectively, had a history of receiving systemic or inhaled corticosteroid before admission; 52 (20.3%) patients received systemic corticosteroid during hospitalization, and 16 (38.1%) received a prednisone equivalent >0.3 mg/kg/day.

In contrast to what is seen in neutropenic patients and stem cell transplant recipients, the relatively typical symptom of fever was present in only two patients.[19] Chest pain and hemoptysis also were present in only two IPA patients. Thoracic CT scan was performed on 4 IPA patients and 102 non-IPA patients, but the diagnostic signs—the halo sign or the air-crescent sign—were only found in only 1 IPA patient. On the whole, there were no significant differences in the CT scan findings of IPA and non-IPA patients. *Aspergillus* culture was positive in four IPA patients and two non-IPA patients (colonization).

Nearly all non-IPA patients (251/254; 98.8%) received antibiotic treatment, with the duration ranging from 5 days to 42 days. Three IPA patients received antibiotic treatment, the mean duration being 12.3 days. Twenty-three non-IPA patients (9.1%) received piperacillin–tazobactam or amoxicillin–clavulanic acid, both of which can interfere with GM detection. Eighteen non-IPA patients (7.1%) had an associated non-aspergillosis infection (usually *Candida*), which can interfere with BG detection. All IPA patients received voriconazole or caspofungin, and three non-IPA patients received anti-*Aspergillus* therapy. The mortalities were 80.0% (4/5) and 5.1% (13/256) in IPA and non-IPA patients, respectively. The main causes of death in these two groups were respiratory failure (IPA: 3/4; non-IPA: 7/13), uncontrolled infection (IPA: 1/4; non-IPA: 4/13) and sepsis (IPA: 0/4; non-IPA: 2/13). The sample size was too small to carry out univariate analysis or multivariable logistic regression analysis. According to the characteristics of the patients, the possible risk factors for IPA in patients with AECOPD were corticosteroid use, mechanical ventilation, and the frequency of hospital admissions in the preceding year (Table 1).

Of 261 samples obtained from the subjects, the test for GM was positive in 20 patients and negative in 241 patients. A GM-positive result was obtained in one patient with possible IPA. The positive predictive value (PPV) and negative predictive value (NPV) were 21.1% and 99.6%, respectively. The test for BG was positive in 48 patients and negative in 213 patients. Both of the two patients with possible IPA had positive results. The PPV and NPV were 8.7% and 99.5%, respectively. There was one proven IPA patient who tested negative for both GM and BG; this patient had received large doses of corticosteroids and short-term anti-aspergillosis therapy before admission.

Most patients without IPA who were false positive for one of the seromarkers tended to be negative for the other one. We examined how combining the results of both tests helped in diagnosis of IPA. IPA was diagnosed only when both seromarkers were positive in any one patient. When both markers were used in combination there was an improvement in diagnostic specificity (Table 2).

DISCUSSION

Assessing the incidence of IPA in relatively less immunocompromised patients (patients with AECOPD for example) is not easy because of the lack of a consistent case definition and the absence of a perfect surveillance system. We used a modified definition of IPA for diagnosis, which resulted in rate of proven or probable IPA in patients with AECOPD of 1.91% (5/261), which suggests the possibility of considering IPA as a differential diagnosis in advanced COPD patients. According to the characteristics of the IPA and non-IPA patients in the present study, the increased susceptibility to invasive *Aspergillus* infection might be caused by long-term or repeated short-term corticosteroid treatment, broad-spectrum antibiotic treatment, and the presence of comorbidities such as diabetes mellitus and renal inadequacy.

During the acute exacerbation period of COPD, a systemic or inhaled corticosteroid is recommended to be used to relieve bronchospasm and the systemic

Table 1. Characteristics of the five patients with proven or probable IPA.

Patients	1	2	3	4	5
IPA classification	Proven	Proven	Probable	Probable	Probable
Gender	Male	Male	Female	Male	Male
Age (years)	65	71	73	63	67
Smoker	Yes	Yes	No	Yes	Yes
GOLD classification	Stage III	Stage IV	Stage III	Stage III	Stage IV
Accompanying disease	Hypertension	Renal inadequacy	Diabetes mellitus	None	Heart failure
Steroid treatment at admission	Systemic	Systemic	Systemic	None	Inhaled
Hospital admissions in last year	2 times	1 time	2 times	1 time	3 times
Thoracic CT scan	Infiltration	Halo sign	Cavitation	Infiltration	Not done
Days between admission and confirmed diagnosis	6	5	12	18	16
Ventilation	Invasive	Invasive	Invasive	None	Invasive
WBC count	14.3×10^9/L	9.1×10^9/L	13.6×10^9/L	8.6×10^9/L	12.7×10^9/L
Sampling methods for *Aspergillus*	Bronchial biopsy by bronchoscopy	Lung biopsy by percutaneous lung puncture	Culture from sputum	Culture from LRT	Culture from LRT
Outcome	Death	Death	Death	Survival	Death

Table 2. Performance of galactomannan [GM] antigen and 1,3-β-glucan [BG] and the combination in detection of invasive aspergillosis.

Test	No. of results				Sensitivity	Specificity
	True positive	True negative	False positive	False negative		
GM detection	4	239	15	1	80%	94.1%
BG detection	4	212	42	1	80%	83.4%
Combination	4	251	3	1	80%	98.8%

inflammatory response syndrome.[20] However, corticosteroids are a double-edged sword since they can impair immune function and have been shown to directly stimulate the growth of *A fumigatus* in vitro.[21] Some reports have suggested that not only systemic, but inhaled corticosteroids might also promote IPA in COPD patients.[22] One patient diagnosed as probable IPA in the present study had received only inhaled corticosteroids. The present study also showed that the frequency of administration of systemic corticosteroids in IPA patients was much higher than in non-IPA patients (100.0% vs. 20.3%), as was the dosage of corticosteroid. This indicates that under certain circumstances clinicians should use corticosteroids with caution.

The clinical picture of IPA in patients with AECOPD was very nonspecific. The signs and symptoms suggestive of angioinvasion were usually absent. In the present study more than half of the cases did not have fever. Of the IPA patients who underwent thoracic CT scan, only one patient had the halo sign, which is consistent with the reported lower sensitivity (5%-24%) of the halo and air crescent signs in non-neutropenic patients in the literature.[23] The absence of the halo sign in this population had no negative predictive power, and if the signs were present, they were far less specific.

The mortality of IPA patients in the present study reached 80%, whereas the mortality was only 5.1% among non-IPA patients, which is in accordance with the findings of Rello et al.[4] The high mortality urged us to find a practical method for early diagnosis of IPA in patients with AECOPD. GM and BG are polysaccharide fungal cell wall components that are released during tissue invasion and can be detected in specimens of body fluids.[24] They are considered useful surrogate markers for invasive fungal infection.[14] The overall sensitivity of both GM and BG in the present study was 80%, which was in agreement with the result reported in literature.[15] The lower PPV and higher NPV in the present study compared with other studies were caused by the relatively low prevalence of IPA in the population with AECOPD. However, the use of GM and BG detection in clinical practice is hampered by high rates of false positive results. In our study, there were 15 and 42 false-positive results with GM and BG, respectively. False-positive BG reactions were associated with fungal infection (non-aspergillosis), the use of albumin, hemodialysis, and certain bacterial infections.[25] False-positive reactions with GM might be caused by the use of piperacillin-tazobactam, amoxicillin-clavulanic acid, or antifungal drugs.[9] A strategy to overcome the inherent deficiencies of these serological tests could be the use of a combination of the surrogate markers of IPA. The results of this study suggest that the combination of BG and GM detection for confirming the existence of IPA was more specific than either test used alone. The value of a combination of GM and BG detection deserves further study.

In conclusion, IPA in patients with AECOPD is an emerging problem. The use of corticosteroids plays an important role in its pathogenesis. IPA poses a major threat to this group of patients, and the associated mortality can be devastating. The combination of BG and GM detection is useful for identifying the false-positive reactions by any one test used alone. The value of this approach needs to be proven in future trials.

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original article

Recombinant activated factor VII in controlling bleeding in non-hemophiliac patients

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BACKGROUND: There have been recent reports on the successful use of recombinant factor VIIa (rFVIIa) in non-hemophiliac patients who have experienced heavy blood loss due to trauma with extensive organ damage and who have received multiple blood transfusions with hemostatic changes without success. The timing of administration, dosage, mortality, units of blood transfusion saved, risk of thrombotic events, and the risk/benefit ratio are still poorly defined.

PATIENTS AND METHODS: We conducted a retrospective review of all medical records of patients who received rFVIIa between January 2003 and March 2008. Data collection included demographic characteristics, diagnosis, indications, comorbidities, and amount of blood products used with rFVIIa, dose of rFVIIa, mortality, and adverse events.

RESULTS: We identified 45 patients, 27 (60%) males and 18 (40%) females, with a median age of 52 years. The median dose of rFVIIa was 40 μg/kg (range, 20-120 μg/kg). Five (11.1%) patients needed a second dose of rFVIIa (dose range of 20-85 μg/kg) whereas three patients (6.7%) needed a third dose of rFVIIa (dose range of 40-60 μg/kg). There was a marked and significant reduction in transfusion requirements for packed red blood cells (P=.0078). Overall transfusion requirements significantly decreased after the infusion of rFVIIa (P=.0323). Nineteen patients (42.2%) died and thrombosis was documented in 3 patients (6.7%).

CONCLUSION: Use of rFVIIa should be based on sound clinical evidence to balance the risks, benefits, and cost if used among non-hemophiliacs. Prospective randomized studies are needed to investigate the efficacy and cost-effectiveness of rFVIIa for this indication and to allow a final assessment of the importance of this treatment.

Recombinant factor VIIa (rFVIIa, Novo Seven, Novo Nordisk, Denmark) is a vitamin K-dependent glycoprotein that is an analog of the naturally occurring protease. In 1999, the US Food and Drug Administration approved its use for the treatment of bleeding episodes in patients with hemophilia and in patients with factor VIII or factor IX inhibitors .[1-3] The success of rFVIIa in controlling hemophiliac bleedings has led to its use for other serious bleeding episodes, such as in non-hemophiliac patients with active bleeding and as a prophylactic agent for high-risk patients with obstetrical bleeding,[4-8] hemorrhage associated with warfarin toxicity,[9,10] intracerebral hemorrhage,[11-12] upper gastrointestinal bleeding,[13,14] intractable bleeding after cardiopulmonary bypass in children and adults and Glanzmann's thrombocytopenia.[15-18]

Several studies have reported the "off-label" use of rFVIIa.[5-8,10-15,17,18] Only two randomized, placebo-controlled, double-blind trials were conducted to evaluate the efficacy and safety of rFVIIa among trauma patients with massive bleeding.[19] The rest are case reports and studies in a small number of subjects. Due to its mechanism of action, rFVIIa can lead to thrombotic complications such as myocardial and cerebral ischemia,[20, 21] deep venous thrombosis or pulmonary thromboembolism.[10,15,22,23] The rate of thromboembolic adverse events (TAEs) related to the use of rFVIIa in hemophiliacs has been 5% to 7% since its introduction.[4,9,24-29] This is largely attributed to its action on activated platelets at sites of bleeding only, and the lack of a systemic effect. A recent review of submissions to the US FDA Adverse Event Reporting System suggested that the risk of TAE and death in nonhemophiliac recipients of rFVIIa was 7% to 15%.[30] Adverse events reported to the FDA suggested that most thromboembolic events were associated with the use of higher doses of rFVIIa and for "off-label" indications, resulting in serious morbidity and mortality.[30]

Early experience and dose finding trials of rFVIIa have suggested that a dose of 90 to 120 μg/kg confers adequate hemostatic effect for most surgical and non-surgical bleeding.[27] Doses of 60 to 90 μg/kg have been used with efficacy between 80% and 87% in serious bleeds and 91% and 94% in surgical bleeding.[26] Incremental doses of rFVIIa have been investigated by various workers to determine if such a strategy leads to better control of bleeding with 71% to 84% effective response.[24,26,28] The data on megadosing, however, need to be investigated further. Pre-existing risk factors for thromboembolic events were strongly associated with adverse events reported with the use of higher doses of rFVIIa and for "off-label" indications. In the most compelling evidence of possible thromboembolic risk associated with rFVIIa, serious thromboembolic event rates of 7% (mainly myocardial and cerebral infarction) were reported in a randomized placebo-controlled study of rFVIIa for intracerebral hemorrhage involving 399 patients.[31] As larger doses and more intensified regimens are being studied for hemophiliacs, thrombotic risks with rFVIIa need to be better characterized. "Safe" dosing regimen should be well defined, especially among nonhemophiliacs and older patients. At present, high-quality randomized studies on the use of rFVIIa in the setting of uncontrolled massive bleeding remain scarce. Hence, a consensus is needed to guide physicians in their practice to make critical decisions in this regard. The questions still remain: Is rFVIIa safe? How effective is it in controlling massive uncontrolled bleeding? Do the benefits outweigh the risks?

PATIENTS AND METHODS

We reviewed the records of all nonhemophiliac patients from King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia who received rFVIIa to control bleeding between January 2003 and March 2008 and were on follow-up for up to 3 months after the infusion of rFVIIa. Patients included in the study were adults (age > 17 years) who had bleeding that could not be controlled by conventional transfusion therapy. Data collection included demographic characteristics (age, sex, weight), diagnosis, co-morbidities, cause of bleeding, mortality, cause of death, dose of rFVIIa, medication (anticoagulation or antiplatelets before bleeding), transfusion data (amount of RBCs, fresh-frozen plasma [FFP], platelets, cryoprecipitate before and up to 48 hours after infusion of rFVIIa), and surgical data (type and indication of operation, re-operation and during operation). Adverse events such as TAEs were recorded. Ethical approval was obtained from the Ethics Committee of King Saud University College of Medicine and Research Center, Riyadh, Saudi Arabia prior to the conduct of the study.

Statistical analysis was done with the Statistical Package for Social Sciences volume 16.0 (SPSS Inc., Chicago, Illinois, USA). Demographic characteristics and baseline characteristics are expressed as median values and ranges. The Mann-Whitney test was used to evaluate the differences and compare transfusion data before and after administration of rFVIIa. The Pearson correlation was used for association between variables. Significance was assumed at $P<.05$.

RESULTS

Of 65 patients who received rFVIIa from January 2003 to March 2008, 20 patients were excluded of whom 18 were hemophiliacs and two were children. In total, 45 patients were included in the analysis. Twenty-seven (60%) were male and 18 (40%) female and the median age was 52 years (range, 17-79 years). None of the 45 patients had any of the registered indications for the use of rFVIIa (hemophilia A or B with inhibiting antibodies, FVII deficiency or Glanzmann thrombasthenia). Bleeding was post-CABG (n=28 patients; 62.2%), gastrointestinal tract (n=5, 11.1%), post-partum (n=5, 11.1%), central nervous system (n=3, 6.7%) and other surgical procedures (n=4, 8.9%). The median APACHE score was 10 (range, 2-33) (Table 1).

The median frequency of rFVIIa administration was two doses per patient (range, 1-4) and the median dose of rFVIIa was 40 μg/kg (range, 20-120 μg/kg). Prior to infusion of rFVIIa, 31 patients (68.9%) were given FFP and, if necessary, platelet transfusion (n=27, 60%), packed red blood cells (PRBC) (n=27, 60%), and cryoprecipitate (n=18, 40%). Bleeding was stopped completely in 28 patients (62.2%) whereas 17 (37.8%) patients needed additional blood products after the initial rFVIIa dose. Five patients needed a second dose of rFVIIa (median dose, 40 μg/kg) and three patients needed a third dose of rFVIIa (median dose, 60 μg/kg).

Transfusion requirements before and in the first 48 hours after the administration of rFVIIa are shown in Table 2. There was a marked reduction in transfusion requirements for PRBCs, from a mean of 9.5 units before rFVIIa to a mean of 5.6 units after rFVIIa. However, no reductions were observed in the transfusion require-

Table 1. Characteristics of rFVIIa use at King Khalid University Hospital, Riyadh.

No. of patients	45
Age, median (range) (years)	52 (17-79)
rFVIIa dose in µg/kg, median (range)	40 (20-120)
APACHE score, median (range)	10 (2-33)
No. of patients who received transfusion prior to rFVIIa	31 (68.9%)
No. of patients who received transfusion after rFVIIa	17 (37.8%)
No. of patients who received additional doses of rFVIIa	8 (17.8%)
No. of patients with control of bleeding	28 (62.2%)
Thrombosis	3 (6.7 %)
Death	19 (42.2%)

Table 2. Differences in transfusion requirements before and after infusion of rFVIIa.

Transfusions	Mean before rFVIIa	Mean after rFVIIa	Mean difference
No. of units packed red blood cells	9.5	5.6	3.9
No. of units fresh frozen plasma	5.6	5.0	0.6
No. of units platelets	7.1	6.6	0.5
No. of units cryoprecipitate	6.7	4.6	2.1
No. of units for total transfusion	28.9	21.8	7.1

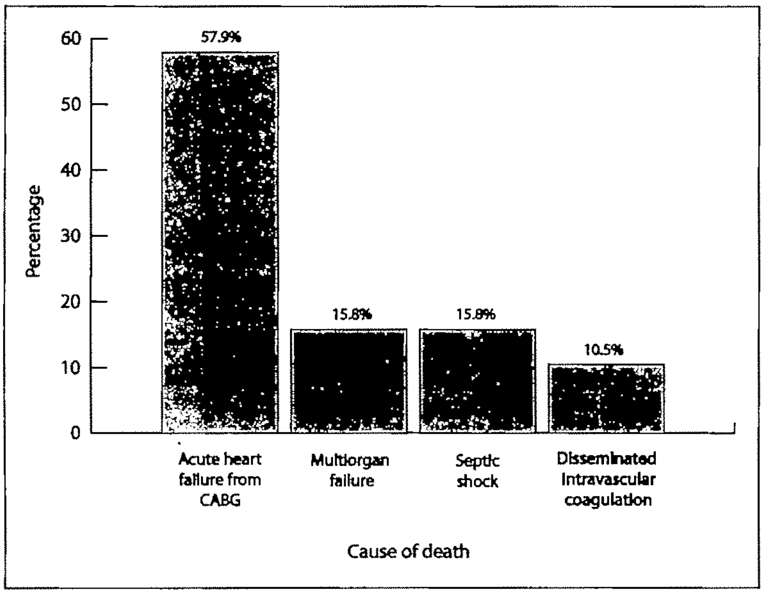

Figure 1. Cause of death among non-hemophiliacs receiving rFVIIa.

ments for FFP, platelets, and cryoprecipitate. Overall transfusion requirements decreased after the infusion of rFVIIa.

Nineteen patients (42.2%) died of which 11 (57.9%) were male and eight (42.1%) were female. The median age of the patient population in this study was 51.5 years (range, 17-76 years) and the median APACHE score was 18 (range, 9-33). The median dose of rFVIIa administered was 45 µg/kg (range, 20-120 µg/kg). Thirteen patients (68.4%) were given FFP, 12 (63.2%) were given platelets, 12 (63.2%) were given PRBC, and nine (47.4%) given cryoprecipitate. Eleven patients (57.9%) died of acute heart failure from CABG, three (15.8%) of multi-organ failure, three (15.8%) of septic shock, and two (10.5%) of disseminated intravascular coagulation (Figure 1). Thrombosis was documented in three patients (6.7%): two males and a female. The median age was 49 years (range, 21-60 years). Acute DVT occurred in the right superior femoral and popliteal veins in a patient one week after rFVIIa infusion, two patients had a cerebrovascular accident, one occurred ten days after rFVIIa infusion and the other three weeks after rFVIIa infusion. The median APACHE score was 16.5 (range, 2-19). The cause of bleeding was post-cardiac surgery in all three patients. The median dose of rFVIIa administered was 30 µg /kg (range, 20-40 µg/kg.) All three patients died of acute heart failure post-CABG.

Patients who died had received a higher median dose of rFVIIa (60 µg/kg vs 25 µg/kg) and were older (58 years vs 53 years) than those who lived. Massive bleeding was documented to a greater extent among patients who died despite the transfusion of additional blood products compared to those who survived (26.3% vs 10.0%).

DISCUSSION

In this study, the mortality rate due to rFVIIa use among non-hemophiliacs was higher than that reported elsewhere.[29,30] This could be explained by the demographic characteristics of our patients (as evidenced by the higher APACHE score of those who died), the inherent impending mortality of the patient's condition, the dose of rFVIIa, the late infusion of rFVIIa, the use of transfusion products, and several other factors such as age, the presence of co-morbid conditions, and electrolyte disorders. Despite higher doses of rFVIIa, massive blood transfusion, and even additional doses of rFVIIa and blood transfusion, bleeding could not be controlled, especially among our patients who have APACHE scores more than 15. However, our study suggests that the infusion of rFVIIa may significantly reduce transfusion requirements and may be beneficial to patients. The transfusion requirement of PRBC and the overall transfusion requirements were significantly reduced in the observation period after rFVIIa administration, compared to before rFVIIa administration by as much as 41% for PRBC and 24.6% for overall transfusion.

Of 19 deaths, 11 (57.9%) were a result of acute heart failure from cardiac surgery. Excessive intraoperative bleeding remains a major complication following cardiac surgery, resulting in increased morbidity and mortality. The massive bleeding associated with the disruption of coagulation mechanisms of the body is such that no amount of blood transfusion can benefit the patient without proper monitoring of the coagulation profile. The principal cause of non-surgical, hemostatic, peri-operative bleeding is a pre-existing, undetected bleeding disorder that could be related to the nature of the operation itself or that arises from coagulation abnormalities due to massive blood loss. Despite several reports claiming the benefits of using rFVIIa in postoperative cardiac surgery bleeding,[15-18] most are case reports or reports from small number of subjects, and hence, the risks and benefits of rFVIIa remain unclear.

Our rate of thrombotic events was 6.7% is in accordance with a 2006 report[30] that stated that most of the thromboembolic AEs follow the unlabeled use of rFVIIa. Our results are in accordance with the recent meta-analysis report by Hsia[29] who reported that mortality was 15% among 3184 nonhemophiliac patients and 7.8% had TAE. Our study showed 42.2% mortality and a 6.7% incidence of TAE.[15,18] Very often these adverse events and mortality are due to the combination and coexistence of various pathologies. Identifying patients at risk remains a major component of preventing excessive blood loss. Understanding the hemostatic changes occurring in the intra- and perioperative period, especially in complex procedures like cardiopulmonary bypass and orthotopic liver transplantation, is crucial in developing new strategies for the management of peri-operative bleeding. This is alarming in the sense that almost half of our patients died despite 68.9% of our patients receiving additional blood products prior to the administration of rFVIIa and even higher doses of rFVIIa.

The effectiveness of rFVIIa in controlling bleeding in 28 of our patients and the observed mortality in 19 patients may have been influenced by the timing of dosing, the interval of administration, and the underlying cause of bleeding. As this was not a controlled trial, we cannot be certain that the reduction in transfusion requirements can be attributed to rFVIIa alone as several other factors such as the patient's condition (low APACHE score), age, and even the absence of co-morbid conditions could have an effect on the survival of these patients. The effect of rFVIIa may be enhanced if it is given early in the course of blood loss. Significant delay in the use of rFVIIa can be avoided because a temporary reduction in bleeding does not reduce mortality.[26,28,32,33] The administration of rFVIIa could potentially lead to thromboembolic events as seen in three of our patients.

These findings lead us to suggest the use of rFVIIa in specific situations such as in younger patients (<50 years old), in patients with low APACHE scores, in patients whose electrolyte imbalances have been corrected, and in patients whose coagulation profiles have been determined and addressed. Inadvertent use of rFVIIa in patients with high APACHE scores (>15), older patients, and in those with co-morbidities, may be futile considering the high cost of the drug and its associated risks and mortality. Prescribing rFVIIa in non-hemophiliacs should be based upon high-quality clinical evidence so as to balance the risks, benefits, and costs. This evidence, however, is scant for clinicians at the bedside or for hospitals that bear the economic consequences of prescriptive decisions. This is more so in a set-up where the guidelines are not formulated nor are the prescriptions of rFVIIa regulated.

Prospective randomized studies are needed to investigate the efficacy, safety and cost-effectiveness of rFVIIa to allow a final assessment of the importance of this treatment for use in non-hemophiliacs.This would help to evaluate the potential hemostatic benefits and adverse effects of rFVIIa. Until prospective, randomized, controlled data are available on the use of rFVIIa, especially in the control of massive bleeding among non-hemophiliacs, we recommend close monitoring of coagulation parameters before, during, and after therapy, especially among high-risk patients (with high APACHE scores, old patients >50 years old, and with significant co-morbidities).

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original article

Prevalence of overweight and obesity in Saudi children and adolescents

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BACKGROUND AND OBJECTIVE: There is limited information on overweight and obesity in Saudi children and adolescents. The objective of this study was to establish the national prevalence of overweight and obesity in Saudi children and adolescents.

METHODS: The 2005 Saudi reference data set was used to calculate the body mass index (BMI) for children aged 5 to 18 years. Using the 2007 WHO reference, the prevalence of overweight, obesity and severe obesity were defined as the proportion of children with a BMI standard deviation score more than +1, +2 and +3, respectively. The 2000 CDC reference was also used for comparison.

RESULTS: There were 19317 healthy children and adolescents from 5 to 18 years of age, 50.8% of whom were boys. The overall prevalence of overweight, obesity and severe obesity in all age groups was 23.1%, 9.3% and 2%, respectively. A significantly lower prevalence of overweight (23.8 vs 20.4; $P<.001$) and obesity (9.5 vs 5.7; $P<.001$) was found when the CDC reference was used.

CONCLUSIONS: This report establishes baseline national prevalence rates for overweight, obesity and severe obesity in Saudi children and adolescents, indicating intermediate levels between developing and industrialized countries. Measures should be implemented to prevent further increases in the numbers of overweight school-age children and adolescents and the associated health hazards.

Obesity-associated diseases are now reported with increasing frequency in obese children and adolescents. Among these diseases are impaired glucose tolerance, type 2 diabetes mellitus,[1-3] and cardiovascular diseases,[4] along with other problems such as impaired quality of life,[5] poor self-esteem,[6] and depression.[7] Furthermore, overweight preschool children are more likely to be overweight schoolchildren,[8] and obese children and adolescents are more likely to become obese adults with all the associated health hazards.[9,10]

Surveillance data indicate a high prevalence of overweight and obesity in children from both developed and developing countries. In the United States, the prevalence of overweight (defined as a body mass index [BMI] >85th percentile) and obesity (defined as a BMI >97th percentile) was 31.9% and 11.3%, respectively, for the age group 2 to 19 years of age in the period of 2003-2006 (the prevalence of BMI >95th percentile was 16.3%).[11] Similarly, 1996 reports from the United Kingdom indicated a 17% prevalence of obesity (BMI >95%) in 15-year-olds,[12] but in 2004, prevalence rates of 24% and 26% were reported in 11- to 15-year-old boys and girls, respectively.[13] Reports from developing countries show disparity in the prevalence of overweight and obesity. A high prevalence, similar to that in developed countries, has been reported from countries like Mexico,[14] Brazil,[15] Qatar,[16] the United Arab Emirates,[17] and Kuwait.[18] In contrast, a low prevalence has been reported from India (4.9% in 2003 and 6.6% in 2005 for overweight)[19] and Yemen (6.2% for overweight and and 1.8% for obesity in 2002-2003 for school children).[20] In Saudi Arabia, reports on the prevalence of overweight and obesity were limited to male school children and a single city.[21-23] To our knowledge, there is no information on the national prevalence of overweight and obesity in Saudi children and adolescents. The objective of this study was to establish the prevalence of overweight and obesity in a representative sample of Saudi school-age children and adolescents.

METHODS

The prevalence of overweight and obesity in Saudi school-age children and adolescents (5-18 years of age) was calculated from the 2005 growth charts data set from the children and adolescents survey.[24] Briefly, multistage probability sampling procedures were used to randomly select a cross-sectional sample from a stratified listing based on the population census in Saudi Arabia that was available at the time of the study design. Therefore, the sample was representative of all the socioeconomic strata. Data collection was made by house-to-house visits and a survey questionnaire, clinical examination and body measurements were completed by primary care physicians and nurses. Weight and stature measurements were performed by trained physicians and nurses according to recommended standards.[25] BMI was calculated according to the formula (weight/height^2). Percentiles and z scores for age were constructed and smoothed out using the LMS methodology.[26-28] The BMI distribution for Saudi children and adolescents allowed for the calculation of the proportion of children and adolescents whose BMI was above the upper cut-off values.[29] The prevalence of overweight and obesity were defined using the World Health Organization (WHO) cut-off values as the proportion of children whose BMI for age was above +1 and +2 standard deviation scores (SDS, z scores) respectively. In addition, the prevalence of severe obesity (BMI >+3 SDS) was calculated to estimate the proportion of affected children and adolescents who might be candidates for bariatric surgery. In addition to the 2007 WHO reference, prevalence data were calculated using the widely used 2000 Center for Disease Control (CDC) reference for comparison. All calculations were performed using the software published by the WHO.[30] Similar calculations were performed using the CDC software whose cut-off values were BMI >85th percentile for overweight and >95th percentile for obesity.[31] The chi-square test was used to compare proportions and $P<.05$ was considered statistically significant.

RESULTS

The national sample size in the Saudi reference was 19 317 healthy children and adolescents from 5 to 18 years of age, 50.8% of whom were boys. The prevalence of overweight, obesity and severe obesity in all age groups was 23.1%, 9.3% and 2%, respectively, with boys having a significantly higher prevalence of obesity (10.1% vs 8.4%; $P<.001$) and severe obesity (2.3% vs 1.6%; $P<.001$) than girls (Table 1, Figure 1). However, girls had a significantly higher prevalence of overweight (23.8% vs 22.4%; $P=.014$). In addition, there was a significantly higher prevalence of overweight (26.6% vs 19.5%; $P<.001$) , obesity (10.5% vs 8.4%; $P<.001$) and severe obesity (2.4% vs 1.5%; $P<.001$) in adolescents than in school-age children. The prevalence of overweight, obesity, and severe obesity in school-age children (5-12 years) was 19.6%, 7.9% and 1.5%, respectively (Table 2). There was no significant difference in the prevalence of overweight between boys and girls (19.9% vs 19.2%; $P=.507$), but boys had a significantly higher prevalence of obesity and severe obesity (9% vs 6.8%; $P<.001$) and (2% vs 1%; $P<.001$) respectively. The prevalence of overweight, obesity, and severe obesity in adolescents from 13 to 18 years of age was 26.6%, 10.6% and 2.4%, respectively (Table 3). More girls tended to be significantly overweight than did boys (28.4% vs 24.8%; $P<.001$), but there was no significant difference between boys and girls in the prevalence of obesity (11.2% vs 10.0%; $P=.101$) and severe obesity (2.6% vs 2.1%; $P=.100$). Tables 4 and 5 show a significantly lower prevalence of overweight (23.9% vs 20.4%; $P<.001$) and obesity (9.5% vs 5.7%; $P<.001$) when the CDC reference was used.

Table 1. Prevalence of overweight and obesity.

Age (years)	BMI > +1 SDS Number of children (%)			BMI > +2 SDS Number of children (%)			BMI > +3 SDS Number of children (%)		
	Boys	Girls	All	Boys	Girls	All	Boys	Girls	All
5-12	6149 (19.9)	5917 (19.2)	12066 (19.6)	6149 (9)	5917 (6.8)	12066 (7.9)	6149 (2)	5917 (1)	12066 (1.5)
13-18	3659 (24.8)	3592 (28.4)	7251 (26.6)	3659 (11.2)	3592 (10.0)	7251 (10.6)	3659 (2.6)	3592 (2.1)	7251 (2.4)
Overall	**9808 (22.4)**	**9509 (23.8)**	**19317 (23.1)**	**9808 (10.1)**	**9509 (8.4)**	**19317 (9.3)**	**9808 (2.3)**	**9509 (1.6)**	**19317 (2.0)**

SDS: standard deviation scores

DISCUSSION

Prevalence data are important for the surveillance of overweight and obesity, provided similar cut-off values and references are used consistently. However, there is no consensus on either cut-off values or on the type of reference used to calculate the prevalence of overweight and obesity, which makes it difficult to compare prevalence studies. Until recently, the National Center for Health Statistics/World Health Organization (NCHS/WHO) reference was used,[32] but one of its most important shortcomings was the lack of a BMI reference for children younger than nine years of age. A revision of the NCHS/WHO reference was conducted by the CDC and recommended as a replacement of the older NCHS/WHO reference.[33] Another reference was developed based on the analysis of multinational surveys of a large number of children and recommended by the authors for international use.[34] According to this reference, cut-off points for overweight and obesity in children were obtained by linking the data to the International Obesity Task Force (IOTF) cut-off points for adults (BMI of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity). Finally, the WHO, after reviewing data sets from several populations, found that the NCHS data set was the most suitable for a smooth transition with the 2006 WHO child growth standard curves at 5 years with a good alignment with the IOTF cut-off values at 18 years. Accordingly, the WHO reconstructed the 1977 NCHS/WHO reference using state-of-the-art statistical analysis. The result was the development of the 2007 WHO growth reference, which is recommended for international use.[35]

In this analysis, the 2007 WHO reference was chosen for the calculation of prevalence because of the advantages and the potential to be the future international reference for the surveillance of overweight and obesity. In addition, the 2000 CDC reference was chosen to point out the implication of using this reference on prevalence values and to allow for comparison of our results with others that use it as reference, because it is still widely used in many health institutions around the world. Comparison with the other references suggested for international use was not performed in this study[34] as other reports indicated less sensitivity in the detection of overweight than the 2000 CDC reference.[36,37]

Using the 2007 WHO reference, the national prevalence of overweight and obesity in our population was established. Comparison of prevalence data in this report with those from other populations was possible only if similar references and cut-offs were used. To our knowledge, there are no reports on prevalence using the 2007 WHO reference. Therefore, comparisons were made with prevalence rates in reports from other populations using the 2000 CDC reference with cut-off values similar to those used by the WHO. The higher prevalence of overweight and obesity in our sample using the 2007 WHO reference (23.9% and 9.5%, respectively) than that using the 2000 CDC reference (20.4% and 5.7%, respectively) may be explained by the different characterestics of the two references. This suggests that the CDC reference underestimates the prevalence of overweight and obesity and points out the effect of the type of reference used on prevalence data.

In a report from a representative sample of US children collected between 2003 and 2006 using cut-off values similar to those of the WHO, the prevalence of overweight (BMI >85th percentile) and obesity (BMI >97th percentiles) in children 2 to 19 years of age was 31.9% and 11.3%, respectively.[11] This was much higher than the prevalence rates of 20.4% and 5.7% for overweight and obesity reported in this study. However, the difference is difficult to interpret because of the different age groups between the two studies. In a report from Mexico, in a nationally representative sample of 10- to 17-year-old children collected in the year 2000, there was a higher prevalence of excess weight in girls than in boys (14.3%-19.1% vs 10.8%-16%).[14] Our findings were consistent with those of this report although our prevalence levels were higher (25.1% vs 24.7%) than those reported from Mexico. This difference may be due to time factors (4-5 years' difference in data collection) or a truly higher prevalence in our populations.[14] It seems that the prevalence of overweight in our population is intermediate between those of the US and Mexico. The overall pattern of gender variation in the prevalence of overweight and obesity from 5-18 years of age, indicating a higher prevalence of overweight in girls, is consistant with patterns reported from Mexico, UAE, and Saudi Arabia.[14,17,22] However, the opposite pattern (higher prevalence in boys) was true for the prevalence of obesity and severe obesity.

In conclusion, this report establishes baseline national prevalence rates of overweight, obesity and extreme obesity in Saudi school-age children and adolescents, indicating intermediate levels between developing and industrialized countries. Preventive measures should be instituted by health authorities to prevent further increases in the prevalence of overweight in school-age children and adolescents and the associated health hazards.

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original article

Establishing abdominal height cut-offs and their association with conventional indices of obesity among Arab children and adolescents

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BACKGROUND AND OBJECTIVES: Obesity, particularly childhood obesity is common in the Middle East, but no studies have examined the relationship of sagittal abdominal diameter (SAD) or abdominal height to conventional markers of obesity in this region. This is the first study to document the association of SAD with measures of obesity among Arab children and adolescents.

METHODS: Nine hundred sixty-four Saudi children aged 5-17 years (365 prepubertal, including 146 boys and 219 girls; 249 pubertal, including 125 boys and 124 girls; and 350 postpubertal, including 198 boys and 152 girls) were included in this cross-sectional study.

RESULTS: SAD was significantly correlated with indices of obesity regardless of gender, but was strongest among pubertal boys. The cut-off values were as follows: for prepubertal children, 14 cm (equivalent to 50th percentile among girls and 60th percentile among boys); for pubertal children, 15 cm for girls (30th percentile) and 16 cm for boys (50th percentile), and for postpubertal, 21.5 cm for girls (70th percentile) and 22 cm for boys (80th percentile).

CONCLUSION: SAD is a reliable indicator of visceral obesity among Arab children and adolescents in particular. Prospective studies should be done to determine whether such an association translates to a promising risk factor for hard endpoints such as diabetes mellitus and coronary heart disease.

By definition, obesity is a disproportionate build-up of energy in the form of body fat, which impairs health. The degree of health impairment is determined by the amount and distribution of fat, and the presence of other risk factors. Among the mentioned factors, it is the distribution of fat that serves as key to understanding the relationship between obesity and disease.[1] The significance of body fat distribution in relation to various metabolic diseases is not a new concept. Studies carried out by the late Dr. Jean Vague showed that insulin resistance and hyperinsulinism are the metabolic bases for accelerated atherosclerosis, diabetes, gout, and uric calculi in the android type of obesity.[2] These insights have imposed a need to determine body composition, and the mass and fat tissue distribution.

Body mass index (BMI) and waist-hip ratio (WHR) are two of the most commonly used indices for obesity. In terms of their relation to adverse coronary outcomes, however, WHR is more consistent as an index of abdominal obesity and a stronger predictor of atherosclerotic events compared to BMI[3] in both men[4] and women.[5] A particularly important anthropometric parameter which has been increasingly applied in the recent years is the sagittal abdominal diameter (SAD) (also called abdominal height) measured by calipers constructed by Kahn.[6] It has recently gained attention because of its stronger correlation to cardiovascular diseases compared to the other anthropometric measures.[7] In a recent study by Sampaio et al, SAD was considered to be a valid predictor of visceral abdominal fat with very high reliability (interclass coefficient, 0.99).[8] Although this anthropometric indicator measures only the visceral fat tissue, it gives a very good estimate of metabolic risks because of the type of fat tissue involved.

Few studies have actually linked SAD to other metabolic abnormalities. A recent study by Mukuddem-

Petersen and colleagues concluded that the use of SAD had no advantages over simpler and more commonly used anthropometric measures such as the waist circumference in older men and women.[9] On the other hand, Petersson and colleagues emphasized that SAD identifies insulin resistance, subclinical inflammation, and hyperlipidemia, at least among Swedish women and women of Middle eastern descent.[10] No such study has been undertaken in the Middle East, particularly in Saudi Arabia, where the incidence of obesity, including childhood obesity, and other metabolic risk factors are high. Furthermore, very few studies have been undertaken with regards to SAD among children. Hence, this study aims to establish cut-off points for SAD (values below the cut-off are considered abnormal) among Arab youth and to determine the relationship of SAD to conventional markers of obesity such as BMI, waist, hip circumference, and WHR.

Table 1. Anthropometric characteristics of subjects according to pubertal stage.

Parameter	Prepubertal (n=365)	Pubertal (n=249)	Postpubertal (n=350)
Age (years)			
Girls	7.6 (1.8)[ab]	12.0 (0.8)[b]	15.5 (1.1)
Boys	7.5 (1.8)[ab]	12.2 (0.8)[b]	15.4 (1.1)
Body mass index (kg/m^2)			
Girls	15.7 (3.5)[ab]	18.5 (3.8)[b]	22.2 (5.1)
Boys	15.4 (3.6)[ab]	17.8 (4.5)[b]	20.6 (5.6)
Systolic blood pressure (mm Hg)			
Girls	99.3 (12.3)[ab]	104.3 (10.6)[b]	108.6 (10.4)
Boys	99.4 (8.9)[ab]	99.5 (13.6)[b]	107.8 (9.8)
Diastolic blood pressure (mm Hg)			
Girls	66.1 (7.2)[ab]	68.4 (8.1)[b]	71.1 (8.0)
Boys	65.3 (6.3)[ab]	66.2 (10.5)[b]	70.7 (9.1)
Waist (cm)			
Girls	53.1 (9.8)[ab]	61.8 (10.4)[b]	68.8 (11.9)
Boys	53.3 (9.9)[ab]	63.5 (10.3)[b]	70.3 (12.4)
Hips (cm)			
Girls	63.1 (9.9)[ab]	76.3 (13.4)[b]	87.1 (13.6)
Boys	61.5 (7.9)[ab]	75.1 (10.6)[b]	85.1 (14.9)
SAD (cm)			
Girls	14.2 (3.4)[ab]	17.6 (4.3)[b]	19.5 (4.5)
Boys	13.8 (2.8)[ab]	16.9 (3.90)[b]	19.6 (4.7)

Data are mean (standard deviation); [a]denotes significance compared to pubertal; [b]denotes significance compared to postpubertal; significant at $P< .05$.

METHODS

This cross-sectional study was conducted at the Diabetes and Endocrinology Research Laboratory of King Saud University, Riyadh Kingdom of Saudi Arabia from January 2008 to December 2008. The subjects were Saudi children aged 5-17 years who were recruited randomly by primary care physicians through door-to-door interviews within Riyadh, Saudi Arabia. Recruitment was part of the ongoing biomarker screening of the Ministry of Health in collaboration with King Saud University. Written and informed assent from the children and parental consent were obtained. Ethical approval was obtained from the Ethics Committee of the College of Medicine and Research Center, King Saud University Riyadh, Kingdom of Saudi Arabia.

The 964 subjects included 365 prepubertal (146 boys and 219 girls), 249 pubertal (125 boys and 124 girls), and 350 postpubertal (198 boys and 152 girls) children. Tanner staging was used to assess the pubertal stage of boys and girls by trained physicians during their physical exam. Subjects within stage 1 were assigned to the prepubertal stage, subjects within stages 2-4 to the pubertal stage, and subjects in stage 5 to the post-pubertal stage. Children with acute or chronic medical conditions as well as those with diabetes and/or asthma were not included in the study. A generalized datasheet containing demographic information and consent was given to the subjects with their parents' assistance along with a sheet for anthropometric measurements.

Trained research nurses were assigned to record anthropometric indices. A measurement called percentile of BMI was used to identify overweight and obesity in children and adolescents.[11] The BMI used for this study was gender- and age-specific for children. Measurements included height, weight, waist circumference, hip circumference, and SAD. Body weight in light clothes was measured to the nearest 0.1 kg using a standardized Detecto balance beam scale (Detecto Scale Inc, Brooklyn, NY, USA). Height was assessed to the nearest 0.5 cm using standardized stadiometers (Detecto Scale Inc, Brooklyn, NY, USA). Subjects were asked to stand upright on a flat surface without shoes, with the back of their heels and the occiput on the stadiometer. BMI was calculated as weight (kg) divided by height in squared meters. Waist circumference was measured using a standardized measuring tape to the nearest centimeter, taken midway between the lowest rib and iliac crest, whereas hip circumference was measured at the level of the greater trochanters. Holtain Khan abdominal calipers (Holtain Ltd, Crymych, UK) were used to measure SAD. Each subject was examined in the supine position on a firm examination table. Using sliding calipers with parallel blades, a direct reading could be made between the lower arm (touching the subject's back) and the sliding upper arm (touching the front of the subject's abdomen). The measurement was taken after normal expiration (as instructed by the research nurse) with the subject in a relaxed position, and the caliper not indenting the subject's skin. Systolic and diastolic blood pressure readings were also measured using an aneroid sphygmomanometer (Omron with pediatric cuffs by Kappa Medial LLC, AZ, USA).

The data were analyzed using the Statistical Package for the Social Sciences (SPSS for Windows version 11.5). Data are expressed as mean (standard deviation) or as median and range if not normally distributed. Group comparisons were done using the t test or by the Mann-Whitney U-test if not normally distributed. Frequencies were expressed in percentages. Normalization of BMI was done to calculate z scores. BMI z scores were plotted according to the SAD percentiles of boys and girls that had been stratified to pubertal stage. In essence, the *z* score represents the number of SD units by which a given score deviates above or below the mean score. As zero corresponds to the mean BMI, the percentile SAD value nearest to a *z* score of zero was deemed to be the cut-off value. Simple and partial correlation coefficients were determined and regression analysis done to determine relationships between variables of interest. Significance was set at *P*<.05.

RESULTS

As expected, all parameters of postpubertal subjects were significantly higher compared to their pubertal and prepubertal counterparts, regardless of gender (Table 1). The cut-off values for SAD are shown in red in Table 2, which shows BMI *z* scores relative to SAD percentiles (Table 2). Although there was a significant correlation between SAD and the indices of obesity, regardless of gender, also noteworthy was the very strong association of SAD with BMI as well as waist and hip circumference of pubertal boys (R values: 0.77, 0.75, 0.74; *P*<.001 respectively). Furthermore, there was a weak association between systolic blood pressure and SAD (R = 0.18; *P*<.05) among pubertal boys that was not present in other subgroups (Table 3). This association was lost after adjusting for BMI, waist, and hip circumference. The strongest association of SAD was with BMI, regardless of gender, during the pubertal stage (R2 = 0.45; *P*<.001) (Figure 1). Neither BMI nor SAD values of postpubertal subjects showed a Gaussian distribution (Figure 2), which probably explains why the cut-off points established for both boys and girls in this age group were beyond the 50th percentile. The prevalence of overweight and obesity in both boys and girls which was most evident in the postpubertal group (Figure 3).

DISCUSSION

Anthropometric measures are clinically useful because their collection is noninvasive and cheap. Measures such as BMI are highly associated with cardiovascular disease. On the other hand, measures of abdominal obesity

Table 2. Percentiles of sagittal abdominal diameter (SAD) and corresponding body mass index (BMI) z scores of subjects according to pubertal stage.

SAD (cm) percentile	BMI z scores					
	Prepubertal		Pubertal		Postpubertal	
	Girls	Boys	Girls	Boys	Girls	Boys
10	11 (-0.15)	11 (0.03)	13 (-0.1)	12.7 (-1.07)	14 (-0.46)	14.1 (-0.39)
20	12 (-0.08)	11 (0.03)	14 (-0.24)	14 (-0.45)	15.9 (-0.63)	16 (-0.46)
30	12 (-0.08)	12 (-0.09)	15 (0.03)	15 (-0.25)	17 (-0.04)	17 (-0.33)
40	13 (-0.45)	13 (-0.05)	15.7 (-0.71)	15.2 (-0.56)	18 (-0.13)	18 (0.66)
50	14 (-0.01)	13.6 (-0.49)	17 (-0.3)	16 (-0.04)	19 (-0.4)	19 (0.57)
60	14.7 (-0.68)	14 (-0.02)	18 (0.3)	17 (-0.26)	20 (0.33)	20 (0.51)
70	15.4 (-0.19)	15 (-0.17)	19 (-0.36)	18 (-0.25)	21.5 (0.02)	21 (0.48)
80	16.5 (0.14)	16 (-0.05)	20.6 (-0.05)	19.5 (0.63)	23 (0.26)	22 (0.14)
90	18.3 (1.38)	18 (-0.2)	24 (1.44)	22 (2.08)	25 91.3)	28 (3.9)

Data are SAD (z score). Cut-off values for SAD in red.

Table 3. Pearson correlation coefficients using SAD as a dependent variable among subjects in different pubertal stages.

Parameter	Prepubertal		Pubertal		Postpubertal	
	Girls	Boys	Girls	Boys	Girls	Boys
Age (years)	0.18^a	0.27^b	0.06	-0.05	0.12	0.23^b
BMI (kg/m^2)	0.24^b	0.22^b	0.32^b	0.77^b	0.50^b	0.44^b
Waist (cm)	0.23^b	0.33^b	0.33^b	0.75^b	0.39^b	0.41^b
Hips (cm)	0.30^b	0.44^b	0.31^b	0.74^b	0.34^b	0.12
Systolic blood pressure (mm Hg)	0.11	0.01	0.03	0.18^a	0.06	-0.02
Diastolic blood pressure (mm Hg)	0.12	0.02	-0.01	0.13	0.05	-0.03

^{a}*P*<.05; ^{b}*P*<.001

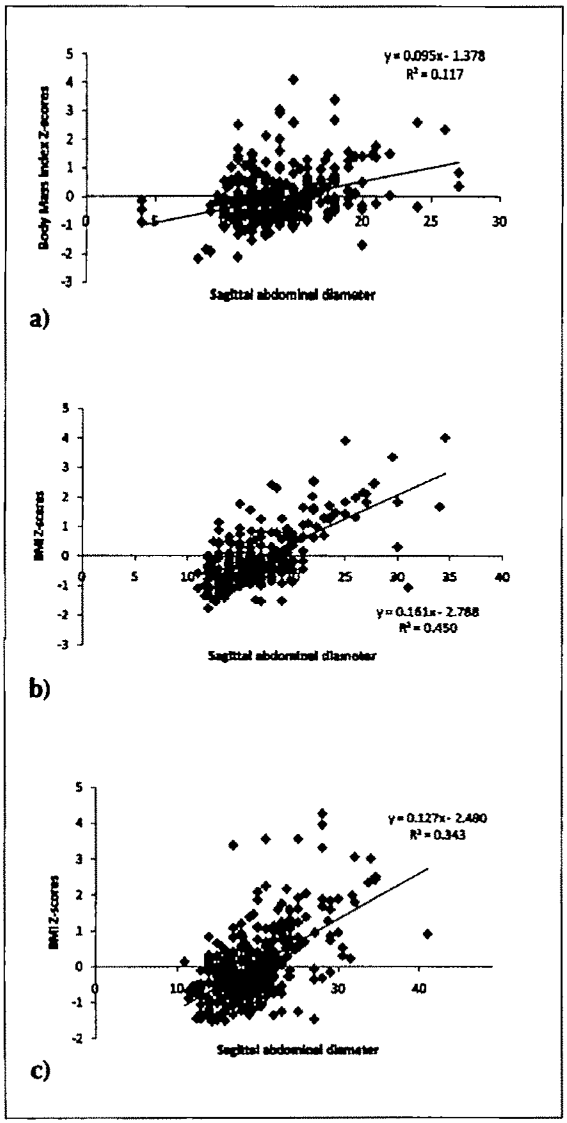

Figure 1. Association of SAD with BMI *z* scores according to pubertal stage: a) prepubertal, b) pubertal and c) postpubertal.

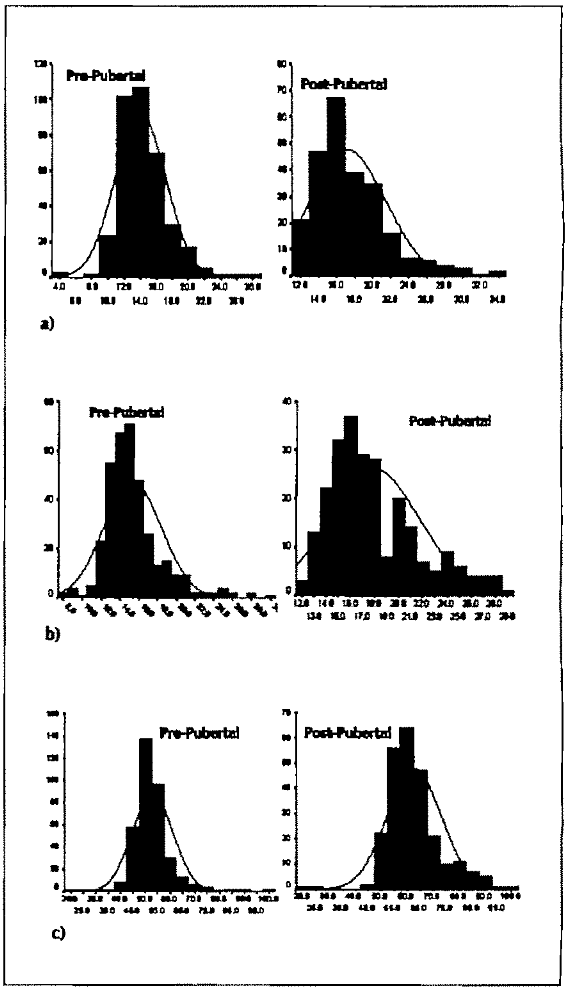

Figure 2. Histograms of selected anthropometric measurements according to pubertal stage: a) SDI Pprepubertal vs postpubertal, b) BMI prepubertal vs postpubertal and c) waist circumference distribution vs postpubertal.

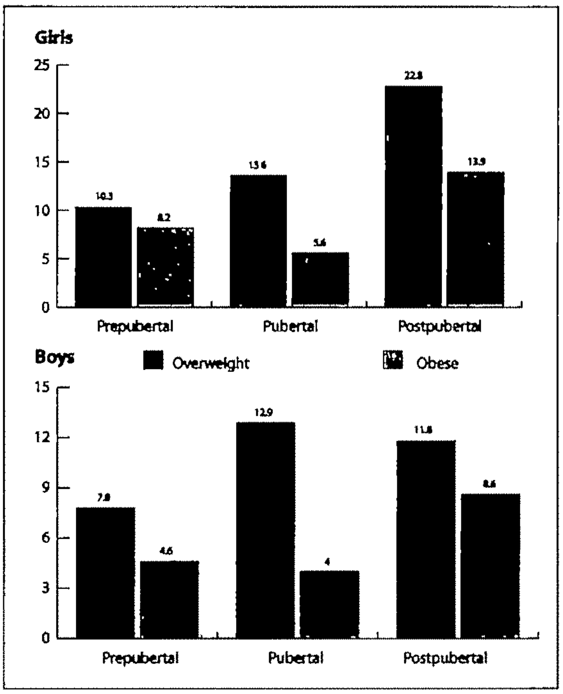

Figure 3. Prevalence of obesity according to pubertal stage.

such as the waist-hip ratio could be more clinically useful than BMI to predict diabetes and cardiovascular disease.[10] SAD has recently gained attention because of its stronger correlation with cardiovascular diseases compared to other anthropometric measures.[11] In a study done by Anjana and colleagues, both waist circumference and SAD were found to correlate well with visceral and abdominal obesity, both of which are strongly correlated to the development of diabetes.[12] In another recent study by Oppert et al, SAD was found to be the only significant predictor of cardiac death compared to other measures of obesity.[13] Kvist et al were the first to demonstrate that the sagittal diameter measured by CT scan was closely related to the volume of visceral fat.[14] The correlation of the sagittal diameter was 0.94 in 19 women and 0.92 in 24 men, with subjects presenting a wide range of BMI. The correlations between the waist circumference and visceral fat were found to be 0.85 and 0.88, respectively. These correlations are considerably higher than those observed between anthropometric variables and the visceral fat area measured at the level of the umbilicus in obese men and women.[15]

To the best of our knowledge, no study has been conducted to determine SAD cut-offs and their relationship to other measures of obesity among Arab youth. This is particularly important as childhood obesity that persists into adulthood is an inevitable factor for the growing incidence of noncommunicable chronic diseases. Furthermore, there is still no existing consensus as to the definition of pediatric metabolic syndrome.[16] Compared to other studies, our postpubertal cut-offs were relatively higher compared to those found in Brazilians (19.3 cm and 20.5 cm as the threshold values for SAD in women and men),[8] suggesting probable ethnic variation or other factors such as choice of the cohort.

In our study, SAD was strongly associated with several components of the metabolic syndrome (BMI and systolic blood pressure) although this does not apply to all ages. This age-wise difference reflects the body's evolution through childhood and puberty and the associated changes in metabolic and clinical characteristics.[16] Nevertheless, SAD remains a promising alternative measure of central obesity among children as it is superior to other anthropometric measures in terms of visceral fat estimation.[17] This is because when a person with enlarged intraabdominal adipose tissue is standing, all fat tissue is pulled downwards. Hence, waist or hip circumference measurements may not be as accurate in assessing the intraabdominal fat, especially among children who are very obese. When the same person lies supine, the mass shifts cranially, causing anterior projection of the abdomen which is measured by the sagittal diameter. Thus, it is the anteroposterior fat that seems to be important for the prediction of the metabolic syndrome.[18]

Our results showed gender differences in terms of the strength of SAD's association with other measures of obesity, which was more evident in the pubertal group as compared to the prepubertal group. This confirms the findings of Arfai and colleagues at least in terms of visceral fat.[19] Furthermore, in our results, boys and girls showed no difference in SAD values, indicating a monomorphic trend in values for intraabdominal adipose tissue at least during childhood. In contrast, visceral fat is more predominant in males due to considerable gender differences in fat topography among adults.[18]

Epidemiological studies conducted in Saudi Arabia point to an increased incidence of overweight and obese Saudi children.[20-24] Given that obesity is an influential risk factor for certain cancers and chronic diseases such as atherosclerosis, hypertension, and most especially, diabetes mellitus (prevalence of 23.7% in 2004 alone[25]), it is imperative to address childhood obesity as critically as one would address its co-morbidities. The use of SAD therefore offers a cheap but reliable index of how close a child is to developing insulin-resistance-related diseases. Establishing SAD cut-offs that are specific to gender and age, is imperative to develop meaningful and evidence-based strategies aimed at reducing risk factors through parental education, school programs, and childhood training.

Although the sample size was large enough to elicit the needed associations, a bigger sample size with the inclusion of rural children would have distinguished the impact of SAD on various age groups as well as establishing more reliable cut-offs that would have been age- and gender-specific. Furthermore, as this study was confined only to anthropometric measurements, further studies which will include other components of the metabolic syndrome such as lipid profile and glucose levels besides other laboratory measurements, are necessary to strengthen the clinical relevance of SAD among the obese pediatric population. A prospective approach is also suggested to determine whether variations in SAD translate to changes in the metabolic profiles of children.

In conclusion, SAD can be used as a reliable indicator of visceral obesity among children and adolescents in particular. Further studies should be done to prospectively compare its association to harder measures such as cardiometabolic risk factors, components of metabolic syndrome, and indices of insulin resistance to determine whether SAD can be a promising risk factor for diabetes mellitus and coronary heart disease.

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perspective

A "good death": perspectives of Muslim patients and health care providers

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BACKGROUND AND OBJECTIVES: Twelve "good death" principles have been identified that apply to Westerners. This study aimed to review the TFHCOP good death perception to determine its validity for Muslim patients and health care providers, and to identify and describe other components of the Muslim good death perspective.

SUBJECTS AND METHODS: Participants included 284 Muslims of both genders with different nationalities and careers. We used a 12-question questionnaire based on the 12 principles of the TFHCOP good death definition, followed by face-to-face interviews. We used descriptive statistics to analyze questionnaire responses. However, for new themes, we used a grounded theory approach with a "constant comparisons" method.

RESULT: On average, each participant agreed on eight principles of the questionnaire. Dignity, privacy, spiritual and emotional support, access to hospice care, ability to issue advance directives, and to have time to say goodbye were the top priorities. Participants identified three main domains. The first domain was related to faith and belief. The second domain included some principles related to self-esteem and person's image to friends and family. The third domain was related to satisfaction about family security after the death of the patient. Professional role distinctions were more pronounced than were gender or nationality differences.

CONCLUSION: Several aspects of "good death," as perceived by Western communities, are not recognized as being important by many Muslim patients and health care providers. Furthermore, our study introduced three novel components of good death in Muslim society.

The basic duty of health care providers is to preserve human health and life, but we should realize that death is the inevitable destiny of mankind predetermined by Allah (the creator of the universe). In cases in which recovery is hopeless, the role of health care providers does not end but rather is modified.[1] Health care providers need to help patients and families minimize suffering and maximize comfort by offering appropriate medical care that is neither excessive nor negligent. This principle cannot be applied in 10% to 20% of cases in which death happens suddenly (myocardial infarction, arrhythmia, intracranial bleeding, aortic dissection, accident), but in 80% to 90% of cases, in which death is predicted,[2] both the patient and the medical team are aware of its imminence. This is known as anticipated death, which must be planned if all goes well.

The whole subject of death and dying has been addressed in the West by pioneers such as Elisabeth Kubler-Ross and Raymond Moody: "Looking deeply into the way that we care for the dying person, they have shown that with unconditional love, and a more enlightened attitude, death can be a peaceful, even transformative experience."[3] However, dealing with a patient at the end of life, aiming to improve quality of life, and planning for end-of-life care requires a good understanding of that patient's state from a medical, social, spiritual, and psychological point of view. To date, this important topic is understudied in schools of medicine, and the extensive literature on medical errors shows that medical experts are rarely blamed for "bad" deaths.[4] The authors of the final report on The Future of Health and Care of Older People (TFHCOP) have identified 12 principles of a good death (Table 1).[5,6]

Most of the conducted studies examined the concept of good death in elderly whites, but no comparable qualitative data exist regarding other races.[7] No qualitative study has been conducted in a Muslim society, and pub-

Table 1. Principles of a end-of-life care according to the report, *The Future of Health and Care of Older People.*

1. To know when death is coming, and to understand what can be expected.
2. To be able to retain control of what happens.
3. To be afforded dignity and privacy.
4. To have control over pain relief and other symptom control.
5. To have choice and control over where death occurs (at home or elsewhere).
6. To have access to information and expertise of whatever kind is necessary.
7. To have access to any spiritual or emotional support required.
8. To have access to hospice care in any location, not only in hospital.
9. To have control over who is present at the time when the end comes.
10. To be able to issue advance directives, which ensure wishes are respected.
11. To have time to say goodbye, and control over other aspects of timing.
12. To be able to leave when it is time to go, and not to have life prolonged pointlessly.

lished papers on this important topic have focused mainly on reviewing Islamic teachings and the literature.[8]

The purpose of this study was to review the TFHCOP perception of good death to determine its validity for Muslim patients and health care providers and to identify and describe other elements for a good death. We tried to elaborate on the concept of good death in our society. The Western definition of "good death" was chosen rather than the Ghanaian or Tibetan one since our medical practice depends mainly on Western references; however, there should be some degree of flexibility in applying the Western perspective since death beliefs have religious and cultural backgrounds.

SUBJECTS AND METHODS

Participants were recruited by a random sampling technique per category from King Abdul-Aziz Military Hospital and from a rehabilitation center and long-stay nursing unit in King Khalid Military Hospital in Tabuk. Since most people die surrounded by medical professionals, even when dying occurs at home, a full spectrum of persons involved with end-of-life care-physicians, nurses, social workers, religious officers, patients with life-limiting diseases, and their home caregivers (family members) were included in the survey.[12,13] Patients and family members were recruited mainly from the Hematology and Oncology Center, especially those with advanced malignancy or those who were under palliative care. Death is a multi-dimensional process that involves physical symptom management as well as certain religious, social, and psychological needs of the dying and their loved ones.[14] Non-physical issues may differ widely, which can be attributed mainly to religious and cultural differences. Therefore, we expected that Muslim participants may perceive these issues in a completely different manner. Approval of the study was obtained from the local research ethics committee.

Each participant was interviewed as well as administered a questionnaire. Before they were administered the questionnaire, they were made privy to the nature of the research and its purpose by asking the following questions: 1) What constitutes a good death and a bad one? 2) Can we develop our own definitions in keeping with our religious principles and cultural values? 3) Is this 12-principle definition valid as a definition of a good death in Islamic culture? 4) Please express your opinions freely. Do not be influenced by others' opinions. 5) Share your own experiences and feelings, and not what you have heard from others. 6) Express your opinions. There is no wrong or right answer. 7) We would like to know your opinions.

We developed a questionnaire on the basis of the TFHCOP's "12 principles of a good death." The questionnaire comprised 12 closed-ended questions to which the participants had to answer in "Yes" or "No." The questionnaires were provided in Arabic or English, depending on the participants' preferences (Appendices 1, 2). To ensure the validity of the questionnaire, before conducting the main interviews, we analyzed the questionnaire in both languages with 10 participants of different subgroups. We ensured that the questions were simple and easy to understand, so that the respondents did not have any difficulty in interpreting and answering the questions. Some participants, however, had difficulty in choosing a reply to a few questions. In such cases, we clarified the questions and explained each option by giving examples. We then let them determine how essential they found the point and to choose their answer accordingly.

We used a qualitative approach to draw out previously unexplored aspects and components of the Muslim perspective. We conducted in-depth, open-ended, and face-to-face interviews and content analysis. We did not impose any theoretical assumptions a priori and instead let the participants suggest any principles that they considered to be essential.

After completing the questionnaire, the participants were asked to express and write their opinions on the principles (not included in the previous 12 points) that constitute a good death. We convened focus groups, each

Table 2. Number and frequencies of responses to each principle by group.

	Total	Sex		Nationality		Career		Patients
		Males	Females	Saudi	Non-Saudi	Health care providers Group 1	Health care providers Group 2	
	284	164	120	147	137	181	77	26
Principle 1: Timing of death	80 (28%)	48 (29%)	32 (27%)	41 (28%)	39 (28%)	50 (28%)	24 (31%)	26 (23%)
Principle 2: Control of what happens	177 (62%)	99 (60%)	78 (65%)	90 (61%)	87(64%)	115(64%)	47(61%)	16 (62%)
Principle 3: Dignity and privacy	271 (95%)	156 (95%)	115 (96%)	144 (98%)	127 (93%)	176 (97%)	71 (92%)	24 (92%)
Principle 4: Pain and other symptom relief	246 (87%)	141 (86%)	105 (88%)	132 (90%)	114 (83%)	163 (90%)	60 (78%)	23 (88%)
Principle 5: Where death occurs	127 (45%)	70 (43%)	57 (48%)	70 (47%)	57 (41%)	86 (48%)	30 (39%)	11 (42%)
Principle 6: Access to necessary information	116 (41%)	50 (30%)	66 (55%)	63 (43%)	53 (39%)	82 (45%)	21 (27%)	13 (50%)
Principle 7: Spiritual or emotional support	255 (90%)	146 (89%)	109 (91%)	135 (92%)	120 (88%)	166 (92%)	66 (86%)	23 (88%)
Principle 8: Access to hospice care	255 (90%)	144 (88%)	111 (93%)	135 (92%)	120 (88%)	165 (91%)	66 (86%)	24 (92%)
Principle 9: Control over who is present	108 (38%)	64 (39%)	44 (37%)	59 (40%)	49 (36%)	71 (39%)	30 (39%)	7 (27%)
Principle 10: To issue advance directives	240 (85%)	139 (85%)	101 (84%)	126 (40%)	114 (83%)	155 (86%)	63 (82%)	22 (85%)
Principle 11: To say goodbye	209 (74%)	119 (73%)	90 (75%)	108 (73%)	99 (72%)	133 (73%)	57 (74%)	19 (73%)
Principle 12: To leave when it is time to go	207 (73%)	110 (75%)	97 (71%)	109 (74%)	98 (72%)	137 (76%)	50 (65%)	20 (77%)

comprising two to seven participants, over a 2-month period. The interviews usually lasted for around 30 minutes per group.

Univariate descriptive statistics (frequencies, percentages) were used to analyze responses to each point of the questionnaire. Percentages are reported as whole numbers. For bivariate analysis, differences in participant characteristics among different questions were determined by using the chi-square test. *P* values of <.05 were considered statistically significant. For new themes we followed a grounded theory approach with a "constant comparisons" method and its related open and axial coding techniques.[9,10] Trustworthiness was noted when participants respond affirmatively to researchers' interpretations.[11]

RESULTS

Researchers interviewed 284 participants. Focus group participants ranged in age from 18 to 71 years (mean age, 37 years). The study sample consisted of 164 males (58%) and 120 females (42%). The study included participants of several different nationalities: 147 Saudi (52%), 42 Egyptian (15%), 23 Syrian (8%), 17 Pakistani (6%), 12 Sudanese (4%), 11 Filipino (4%), 11 Jordanian (4%), 10 Malaysian (4%), 7 Indian (3%), 2 South African (1%), 1 Moroccan (<1%), and 1 Nigerian (<1%). The most common groups among the participants were 93 nurses (33%), 88 physicians (31%), 43 patients' relatives (15%), 26 patients (9%), 16 social workers (6%), 14 clinical pharmacists (5%), and 4 hospital religious officers (1%).

Some focus group members were concerned about our society's tendency to consider death as taboo, something that human beings cannot interfere with. Participants informed us of cases in which health care providers avoided end-of-life discussions because they believed that it is beyond our control as humans.

On average, each participant agreed with the eight principles of the questionnaire, so we selected the top eight as the important principles for our society. Table 2 shows the frequencies and percentage of "yes"

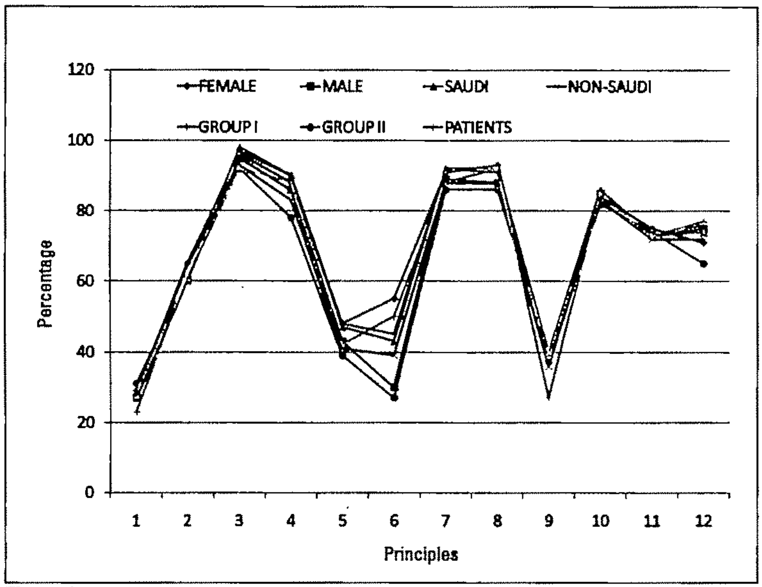

Figure 1. Percentage of responses to each principle by group.

Table 3. Principles of a good death from the Muslim perspective.

· Aspects related to faith and relationship with Allah
· Aspects related to self-esteem and person's image in the eyes of relatives
· Aspects related to concerns about family security
· To be afforded dignity and privacy
· To have access to any spiritual or emotional support required
· To have access to hospice care in any location, not only in hospital
· To be able to have control over pain relief and other symptom control
· To be able to issue advance directives, which ensure wishes are respected
· To have time to say goodbye and control over other aspects of timing
· To be able to leave when it is time to go, and not to have life prolonged pointlessly
· To be able to retain control of what happens

answers for each principle according to nationality, gender, and career. Health care providers were categorized into two groups: Group 1 included health care providers who are involved mainly in medical issues, i.e. physicians and nurses; Group 2 included the other members of the multi-disciplinary care team who are involved mainly in other aspects of care, i.e. patients' relatives, social workers, clinical pharmacists, and hospital religious officers.

Participants identified certain aspects of quality of death that are not mentioned in the Western literature but prove to be essential for Muslims. These can be summarized in three main domains:

Religious faith and beliefs

The first domain is related to a Muslim's faith, belief, and preferences during the dying process. This includes matters like 1) being sure that somebody is there to prompt him with *Shahadah* (bearing witness that there is no true God but Allah and Muhammad is verily His Servant and His Messenger) as a final statement of faith, 2) the presence of someone at the bedside to recite chapters of the Noble Qur'an, 3) to die in a position facing the holy mosque in Makkah, 4) to die in a holy place (e.g., Madinah, Makkah, or mosque) or in a holy time (e.g., in Ramadan or on a Friday).

Self-esteem and body image

The second domain includes some principles related to the patient's self-esteem and image in his friends' and relatives' eyes, by avoiding post-mortem distortions, deformities, septic wounds, or bad odors by maintaining continence and keeping the body and clothes free of urine, stool, and vomit, and making sure the body has a normal appearance after death.

Concerns about family security

The third domain is related to the patient's satisfaction about his relatives. In other words, he needs to feel that his family will be secure and have no trouble after his death, so he will not be worried about them. This relates primarily to economic and social concerns.

It is worth mentioning that data obtained from a sub-group of Saudis reflected the same eight principles selected by major subgroups including females, health care professionals (Group 1), non-medical health care providers (Group 2), patients' home caregivers, and patients themselves (Figure 1). However, the order of sequence of these selected eight points was slightly different. The resultant responses were almost identical to all questions with minimal differences among groups. On the other hand, the three new themes reflect the common ground shared by participants. However, we found remarkable differences among groups. In fact, professional role distinctions were more pronounced than were gender or nationality differences. For example, most physicians and nurses were highly attuned to the needs of good hygiene and preservation of a patient up until his last breath. Religious officers discussed faith and spiritual support and were the only group to discuss postmortem preservation of configuration. Social workers' discussions were more psychosocial in nature and focused on being reassured about the family's future after their death. All themes were present in the patient focus group, but this group showed more concern about faith and their relationship with Allah Almighty.

DISCUSSION

This study reveals that several aspects of "good death" as perceived by Western communities are not recognized as being of special importance by many Muslim patients and health care providers. Furthermore, our study introduced three novel components of good death in Muslim society. The definition of "good death" that is acceptable to Muslim patients and health care providers consists of 11 principles (Table 3). There was some kind of consensus on the importance of dignity and privacy, which can be explained by the Islamic perspective that respects human dignity and privacy and regards each as a fundamental pillar of Shari'a (Islamic Law). Secondly, Muslims by and large value spiritual and emotional support. They also believe that death is closely linked to faith. Therefore, most participants appreciated the importance of access to any needed spiritual or emotional support.

Control over pain and other symptoms is generally required by most people.[14] Muslims perceive suffering as atonement for one's sins. This interpretation helps patients and family members cope with diseases. However, it does not belittle the fact that every effort should be made to relieve suffering. In our study, we met participants who prefer not to receive pain management because they believe that if a patient feels pain and shows patience, he will be rewarded more by Allah and will be more pure. We have to respect this wish because patients are granted the full right to accept or to refuse medical intervention despite the fact that Islamism encourages seeking treatment and there is a lack of religious evidence to support this point of view.[8] Principle 4 may be modified to suit Muslim patients: "To have control over pain relief and other symptom control according to patient desire or preference."

Further, while conducting interviews, we realized that some medical staff, including physicians, are not aware of "advance directives." However, most of the participants preferred to issue advance directives, i.e. the right to refuse any therapeutic intervention, after the concept was explained to them. This study emphasizes that advance directives are underused in the medical practice of this hospital and that we have to encourage adopting it in our hospitals, especially since this study revealed that it is widely accepted by Muslim participants. It is very common in Arabic and Islamic societies to contact relatives and loved ones who may want to see the patient before death, but a physician has to suggest this when he feels that death is imminent and the patient is about to take his last breath. This is also standard practice in the West: staff members invite relatives to be present when death is impending to quietly say their goodbyes.[15] If the number of visitors exceeds the available space, health care providers are expected to express a high degree of sensitivity if it becomes necessary to intervene in such situations.[8]

One cause of increased suffering is facing a lonely death in hospitals surrounded by strangers and advanced technologies and procedures that needlessly prolong the dying process without realizing the fact that the end of life is approaching.[16,17] This may be a good reason why most participants chose to be able to leave when it is time to die and not to have life prolonged pointlessly. More importantly, Muslims do not generally believe that life is pointless, even when it is associated with a significant amount of suffering. They believe that Allah has the ultimate wisdom and is the most merciful and He will reward those who express patience and satisfaction when inflicted with disease or suffering. This, on the other hand, emphasizes the importance of being honest with patients, telling them about their prognosis, and giving them detailed explanations of Do Not Resuscitate orders. This may help in reassuring patients and makes them feel more comfortable that they will not receive any futile intervention.

Muslims believe that it is impossible to tell the time or place of death by any means and that it is only known by Allah Almighty who predetermines the exact timing and place of death. This may explain why the agreement rate for these principles was the least in this study, although many participants confirmed that it would be nice to have a "death alarm" that would enable the person to repent for his sins and ask for forgiveness. This supports the view that the patient and his family prefer to be given a less definitive answer. Surprisingly, if given a choice of a specific place to die, some participants repeatedly stated that they preferred to die in a holy place like a mosque, or in Makkah or Madinah. In this case, health care providers have to respect the patient's wish and may sometimes even be asked by the patient to help in persuading his/her family to grant this wish. This may be achieved by building a good rapport with the family and being honest and open with them regarding the patient's status, prognosis, and goals of care at that stage. This approach may help to decrease the patient's stress and prevent family members from feeling guilty about leaving him to die outside the hospital. Readers may note that we talk about the patient and his family as one unit, which contradicts Western practice and the concept of medical confidentiality. This could be attributed to the distinct nature of the Muslim family and its composite interrelations and strong ties.

This study has some limitations related to method and study design. A "Yes/No" question format may not be the best way to measure participants' preferences in this type of research. A five-point Likert scale would have been a better assessment tool because it would allow the participants to choose from a range of options that reflect the degree to which they agree or disagree with a statement and also to abstain from committing themselves to one response or the other if they were not sure. Although the fact that we obtained similar responses from all subgroups may support test reliability, further studies are required to assess the validity of using a questionnaire for this kind of topic. The questionnaire was provided in one of two languages, which may bear the potential for inter-rater variability and language translation inaccuracy. Researchers conducted a pilot study with 10 participants who received the questionnaire in one language and then in the other language. Proper modifications were made to ensure receipt of the same response. There was a preliminary assumption that principles of good death among whites, as reported by a TFHCOP, are applicable to a group of Muslim participants. This was obvious by adopting these principles as leading questions addressed to Muslim participants. The authors suggest that similar studies can be repeated using modified principles that are in better concordance with Islamic culture. Although our participants represented a broad range of ages, nationalities, careers, and educational levels, all were recruited from Northwest Armed Forces Hospitals; therefore, our findings may not reflect the perceptions in other areas. A large study on a national or international basis may be of importance to address this issue.

Our study has implications for both education and practice. Health care providers must understand their patients' preferences and respect their needs regardless of what the health care provider believes. Physicians and nurses should do their best to care for each patient's appearance and hygiene, as this is of great importance to Muslims. This can be achieved by keeping the clothes and body of the patient free from urine, stool, vomit, or blood whenever possible. They should be ready to help the patient to take a bath more frequently as well. To reduce postmortem disfigurement, jaw fixation and eye closing must be done immediately at the time of death. All patients should be reassured that this is the practice with all deceased Muslims in the hospital. The rites of washing, shrouding, funeral prayers, and burial should follow as soon as possible. In addition to expressing empathy, the major role of the health care team at this stage is timely documentation to prevent any unnecessary delay in proceeding with funeral rites. Physicians should be reminded that they are not alone when caring for dying patients; many other health care providers (nurses, social workers, and religious officers) are available to provide comprehensive care. Physicians may ask a screening question regarding any faith-related concerns and then ask whether the patient or relatives would like to speak in greater depth with a religious officer (a Sheikh or Imam). In addition, the physician should ask the patient a screening question to figure out any concerns related to his relatives and make sure that he has no other worries. The physician can then consult the social worker to help him in this respect. However, the palliative care team may need to identify families with suboptimal resources, provide the necessary support during bereavement, and reassure the patient that his family will receive economic support after his death. This is not the physician's primary duty, yet he should explore this point and ask for proper consultation, if necessary.

It is important that the medical curriculum covers this issue in detail. The Islamic perspective of "good death" must be included in health care services, professional codes, and care plans or missions for end-of-life care organizations and institutions in Islamic countries. This is more complicated than simply translating Western literature and trying to apply it to our practice that exists in a society with different cultural and religious backgrounds; care must be taken to develop such information in a Muslim-centered manner.

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1. أن تعلم أن الموت وشيك أو تعلم المتوقع حدوثه له	لا نعم
2. أن تبقى مسيطراً على مجريات الأمور	لا نعم
3. أن تحظى بشكل يحفظ الكرامة و الخصوصية	لا نعم
4. أن تتلقى علاجاً كافياً للسيطرة على الألم والأعراض الأخرى	لا نعم
5. وتتحكم بمكان حدوث (الموت)البيت أن تختار المستشفى......)	لا نعم
6. أن يمكنك الوصول للخبرات والمعلومات الضرورية من أي نوع	لا نعم
7. أن تستطيع الوصول لأي دعم روحي أو عاطفي	لا نعم
8. الطبية في أي مكان تحتاج له ,, أن تتلقى الرعاية ال وليس في المستشفى فقط	لا نعم
9. أن تتحكم بمن هم الأشخاص الذين سيحضرون	لا نعم
10. أن تتمكن من كتابة توجيهات مستقبلية لما يخص حالتك الصحية ,, وتعلم أنها ستحترم	لا نعم
11. أن يكون لديك الوقت لتوديع الذين تحبهم	لا نعم
12. أن تستطيع مغادرة هذه الحياة في الوقت المناسب	لا نعم

الرجاء كتابة نقطة أو أكثر موجودة في تصورك عن الموت بسلام ,, أو عن الميتة الحسنة لم يتم ذكرها في النقاط السابقة

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Appendix 1. Questionnaire in Arabic.

1. To know when death is coming, and to understand what can be expected.	Yes No
2. To be able to retain control of what happens.	Yes No
3. To be afforded dignity and privacy.	Yes No
4. To have control over pain relief and other symptom control.	Yes No
5. To have choice and control over where death occurs (at home or elsewhere).	Yes No
6. To have access to information and expertise of whatever kind is necessary.	Yes No
7. To have access to any spiritual or emotional support required.	Yes No
8. To have access to hospice care in any location, not only in hospital.	Yes No
9. To have control over who is present and who shares the end.	Yes No
10. To be able to issue advance directives.	Yes No
11. To have time to say goodbye.	Yes No
12. To be able to leave when it is time to go.	Yes No
13. To make peace with others before death.	Yes No
14. To die naturally after a long and well-spent life.	Yes No
15. A good death is a death which is accepted by the relatives	Yes No

Other principles or aspects which is not mentioned above:

Appendix 2. Questionnaire in English: What do you think about these principles? Do you think they are well-matched to your conception of a "good death?"

brief report

Water pipe (shisha) smoking among male students of medical colleges in the eastern region of Saudi Arabia

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BACKGROUND AND OBJECTIVES: Shisha smoking, one of the commonest methods of smoking tobacco among Arabs, increases the risk of cardiovascular and respiratory diseases. The objective of this study was to determine the prevalence of shisha smoking among male students of three colleges and to identify factors associated with shisha smoking.

METHODS: This cross-sectional study included 500 male students of three colleges (medicine, applied medical sciences and dentistry). Data were collected from 371 students using a self-administered questionnaire.

RESULTS: The overall prevalence of shisha smoking was 12.6% (8.6% shisha only and 4.0% both shisha and cigarettes). Thirty students (63.8%) started shisha smoking at ages of 16 to 18 years. Seven students (15%) smoked shisha daily. Cafés or restaurants were the favorite places for smoking (70.2% of students). There was a high prevalence of shisha smoking among students whose mothers had a secondary (19.1%) and higher (53.3%) education.

CONCLUSIONS: There was a high prevalence of shisha smoking among university students. The majority of students started shisha smoking at a young age. Public health measures, including the banning of smoking in public places are recommended.

Shisha smoking (using a waterpipe, narghile or hookah) is a social and entertainment behavior of increasing popularity, especially among adolescents.[1-3] Shisha smoke contains high concentrations of carbon monoxide, nicotine, tar and heavy metals.[4,5] Thus, shisha smokers are at a greater risk of serious respiratory diseases and cancers.[6-9] Shisha smoking is highly prevalent in developing countries and in the Eastern Mediterranean region.[10-12]

In Saudi Arabia there is a recent trend toward increased Shisha smoking.[13-16] Saudi adolescents nowadays spend part of their leisure time smoking shisha in cafes and restaurants. Several studies have shown that shisha smoking is practiced more frequently (either daily or once per week).[17,18] The favorite places for smoking shisha are with friends in café and open places.[16,17,19] The objective of this study was to determine the prevalence of shisha smoking among male students from three medical colleges of King Faisal University in Dammam, Saudi Arabia and to identify factors associated with shisha smoking.

METHODS

This was a cross-sectional study conducted at King Faisal University in Dammam city, Eastern Province, Saudi Arabia during the year 2008. The target population consisted of all male students (levels 1 to 3) of the three colleges namely, medicine, applied medical sciences and dentistry, with 572 students registered for the academic year 2008. Other levels (levels 4 to 6) were excluded because they usually study outside the university campus and were not accessible during the time of the study. The study was approved by the research committee of the College of Medicine and the university authorities. The objectives of the study were explained to the participating students after which they gave their informed consent. The information on individual students was kept confidential. The study sample was calculated by the computer package Epi Info (Epi Info TM version 3.3.2, 2005, CDC, Atlanta, USA) according to the total number of the students with an estimated average prevalence of shisha smoking of 10%[20] at a confidence level of 95%. Thus, an estimated sample size of 500 students was included in the study using a simple random sampling technique. Three hundred seventy-one students responded (a response rate of 74.2%). They were distributed as follows: medicine (n=225 of 322 students), applied medical sciences (n=95 of 167 students), and dentistry (n=51 of 76 students. All Saudi and non-Saudi students were included in the study. A pilot study was conducted to test the validity and the logistics of the study. Subjects selected in the pilot study were excluded.

Data were collected through a self-administered questionnaire constructed by the investigators. It included questions on socio-demographic variables, current shisha smoking, smoking pattern and personal and family factors. Data collected were checked for accuracy and completeness, coded and entered into the SPSS 16 (SPSS Inc., Chicago, Illinois, USA) in a personal computer. Associations were determined by chi-squared test and statistical significance was taken as $P<.05$.

RESULTS

The majority of students were from the College of Medicine (n=225, 60.6%) and were of Saudi nationality (n=367, 98.9%). Approximately half of the students' fathers had a higher education and higher family income. Two hundred sixty-six (72%) students lived with their families. The overall prevalence of shisha smoking was 12.6% (n=47), including shisha only (n=32) and both shisha and cigarettes (n=15). (Table 1). There was a high prevalence of cigarette smoking among fathers (n=51, 13.7%), and 5.4% (n=20) of the students' fathers as well as 2.4% (n=9) of mothers smoked shisha. Twenty-eight students (7.6%) had a shisha smoker in their residence. The majority of university students (n=30, 63.8%) started shisha smoking at the ages between 16 to 18 years, while 8 students (17.1%) started smoking before the age of 16 years (Table 2). Seven students (15%) smoked shisha daily, while 9 (19%) smoked 2 to 3 times per week. Cafés or restaurants were the favorite places for shisha smoking for the majority of students (n=33, 70.2%). Twenty-nine students (62%) spent 1 to 3 hours in shisha smoking per one session. A high proportion of students had the habit of smoking shisha with friends at any time (n=31; 66%), after eating (n=25; 53.2%) and with tea and coffee (n=18; 38.3%). About one-fifth of students smoked shisha during examination times (n=10, 21.3%) and when angry or stressed (n=9, 19.1%).

A significantly higher proportion of shisha smokers were students from the College of Medicine compared with the other two colleges (Table 3). More education among mothers was significantly associated with shisha smoking. Shisha smoking was practiced more in houses where there were no smokers in the residence.

DISCUSSION

The overall prevalence of shisha and cigarette smoking was 12.6% with a prevalence of 8.6% for shisha smoking only. This result was higher than that reported by Almutairi study of 7.3% among university students in Riyadh.[15] It was also higher than the result shown by Abolfotouh et al in Abha (34 shisha smokers out of 489 students).[16] The Al-Turki study conducted in central Saudi Arabia revealed that among medical student smokers, 44.1% smoked shisha and 23.7% smoked both cigarettes and shisha.[21] A high prevalence of shisha smoking was also shown by other studies from the Gulf and Eastern Mediterranean countries.[11,17,19,22] A survey among 937 British university students showed a prevalence of 8.0% for regular waterpipe smokers.[23]

The high prevalence of shisha smoking among King Faisal University students should raise the awareness of public health and administrative authorities about this problem. Approximately 64% of university students started shisha smoking at the ages 16 to 18 years. This result is consistent with the Maziak et al study in which the mean (SD) age of initiation of shisha smoking was 19.2 (2.2) years.[12] It was also consistent with the Mohammed et al study in Kuwait where 30% started shisha smoking at ages 14 to 17 years.[24] These findings show that shisha smoking is becoming more popular among adolescents. The harmful consequences of shisha smoking will soon hit the productive sector of the population. Adding to this, the finding of this study is that shisha smoking was practiced frequently (15% smoke shisha daily, 51% once per week and 19%, 2-3 times per week), which is in agreement with other studies. Other studies have shown that shisha smoking was practiced more frequently.[11,17,18,24] Thus students are more exposed to the inhalation of toxic materials in tobacco. It is therefore a growing and unrecognized public health issue.

Cafés or restaurants were the favorite places for shisha smoking in our study. This result was similar to the Anjum et al[19] and Abolfotouh[16] et al. Several studies have shown that shisha smokers were significantly more likely to have shisha smokers as friends.[12,24] These find-

Table 1. Distribution of the university students (n=371) according to their smoking status.

Characteristics	No.	%
Smoking status		
Non-smokers	313	84.4
Shisha only	32	8.6
Cigarettes only	11	3.0
Both cigarettes and shisha	15	4.0
Father		
Non-smoker	297	80.1
Shisha	20	5.4
Cigarettes	51	13.7
Shisha and cigarettes	1	0.3
Refused to answer	2	0.5
Mother		
Non-smoker	358	96.5
Shisha	9	2.4
Cigarettes	1	0.3
Refused to answer	3	0.8
Presence of smokers in place of residence		
Non-smoker	258	69.6
Shisha	28	7.6
Cigarettes	61	16.4
Shisha and cigarettes	22	5.9
Refused to answer	2	0.5
Smoking wife among married students (n=8)		
Non-smoker	5	62.5
Shisha	1	12.5
Refused to answer	2	25.0

Table 2. Smoking habits of the students (n=47).

	No.	%
Age of starting shisha smoking		
10-12 years	2	4.3
13-15 years	6	12.8
16-18 years	30	63.8
≥18 years	3	6.4
Refused to answer	6	12.8
Frequency of shisha smoking		
Once per week	24	51.1
2-3 times per week	9	19.1
> 3 times per week	2	4.3
Daily	7	14.9
Refused to answer	5	10.6
Time spent in shisha smoking in one session		
<1 hour	13	27.7
1-3 hours	29	61.7
>3 hours	2	4.3
Refused to answer	3	6.4
Favorite places for shisha smoking[a]		
Café or restaurants	33	70.2
Open places	14	29.8
Home	3	6.4
Sports clubs	1	2.1
Any place with friends	20	42.6
Conditions associated with shisha smoking		
Tea and coffee drinking	18	38.3
After eating	25	53.2
When feeling happy	5	10.6
When angry or stress	9	19.1
During examination times	10	21.3
With friends at any time	31	66.0

[a] Response categories were not totally exclusive.

Table 3. Variables associated with shisha smoking status of university students.

Variables	Shisha smokers (n=47)		Non-shisha smokers (n=324)		Total (n=371)		χ^2-test (df)	*P*
	No.	%	No.	%	No.	%		
College								
Medicine	19	40.4	206	63.6	225	60.6	16.6 (2)	<.001
Applied Medical Sciences	13	27.7	82	25.3	95	25.6		
Dentistry	15	31.9	36	11.1	51	13.7		
Mother's education								
Illiterate or read and write	6	12.8	68	21.0	74	19.9	14.1 (5)	.015
Primary	1	2.1	40	12.3	41	11.1		
Intermediate	5	10.6	41	12.7	46	12.4		
Secondary or diploma	9	19.1	77	23.8	86	23.2		
University or higher education	25	53.2	91	28.1	116	31.3		
Refused to answer	1	2.1	7	2.2	8	2.2		
Presence of smokers in place of residence								
Yes	21	44.7	90	27.8	111	29.9	5.6 (1)	.018
No	26	55.3	234	72.2	260	70.1		

ings show the influence of friends and peer pressure on shisha smoking. The attitudes and beliefs of the parents, the availability of recreation facilities and the regulations governing smoking at public places should be considered when planning for intervention strategies.

The higher proportion of shisha smokers among students of the medical college might possibly reflect the higher socioeconomic status of their families. Anjum et al study showed that the highest percentage of shisha smoking was observed among college students in a higher socioeconomic group.[19] The finding that a large proportion of shisha smokers have mothers with higher levels of education was interesting. One explanation is that a highly educated mother might consider shisha smoking prestigous and an indication of a modern standard of living. Our results are in agreement with several studies that showed a high prevalence of shisha and cigarette smoking among students of highly educated parents.[11,19] Further studies are needed to explore the influence of parents' education on smoking. A high proportion of shisha smokers did not have smokers in their residency. The difference was statistically significant. This result was not in accordance with other studies which showed that smoking is associated with the presence of a smoker in the residence.[12,16,24]

A limitation of this study is the response rate (74.2%), which was lower than the calculated sample size. This might be due to the fact that some students preferred not to reveal that they were smokers. In conclusion, this study showed a high prevalence of shisha smoking among male university students with the majority starting smoking at a younger age. The study also revealed the influence of friends and peers on the initiation and continuation of shisha smoking. Public health interventions, including laws and regulations governing smoking at restaurants and cafes, should be implemented.

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Clinical experience with alteplase in the management of intracardiac and major cardiac vessels thrombosis in pediatrics: a case series

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BACKGROUND AND OBJECTIVES: Experience with alteplase in pediatric patients is limited and recommendations are extrapolated from adult data. Comprehensive guidelines on the management of thromboembolic events in this group are lacking. We assessed the efficacy and safety of alteplase (recombinant tissue plasminogen activator) in the management of intracardiac and major cardiac vessel thrombosis in pediatric patients.
METHODS: All pediatric patients, 14 years of age and younger, with intracardiac or major cardiac vessel thrombus who were treated with alteplase from 1997 to 2004 at our tertiary care institute were identified through the pharmacy database. Patient data were retrospectively evaluated for the efficacy and safety of altepase.
RESULTS: Five cases were eligible out of nineteen who received alteplase. Patient ages ranged from 40 days to 13 years. The initial dose of alteplase ranged from 0.3 to 0.6 mg/kg followed by a continuous infusion in three patients with a dosage range between 0.05 and 0.5 mg/kg/hr, while intermittent infusion was used in the other two patients. The duration of therapy ranged from 2 to 4 days. By the end of the treatment, two patients had complete resolution of thrombus and one had partial resolution. Two patients failed to respond and had "old" thrombus. Major bleeding events were reported in three patients. The rest had minor bleeding events.
CONCLUSION: Alteplase may effectively dissolve intracardiac thrombi, particularly when freshly formed. Continuous infusion for a long duration appears to be associated with an increased risk of major bleeding. Optimal dose and duration of infusion are still unknown.

Central line and congenital heart disease appear to be the most important associated risk factors for thrombosis in neonates and children.[1] The risk of acute thrombosis in catheterization procedures reaches 10%.[2] The incidence of clinically apparent thrombosis in neonates (excluding stroke) was 2.4 per 1000 from the Canadian registry and 5.1 per 100 000 symptomatic thromboses as recorded in the German registry.[3] Arterial thrombosis occurred in 34% and 24% of total thrombotic events in the Canadian and German Registry, respectively. Although the incidence is relatively low, its associated complications can be serious and fatal. Mortality of thrombotic events was 16% in children due to pulmonary embolism and deep vein thrombosis.[3] Associated morbidity was also high; 8.1% of patients had recurrent thrombosis and 12.4% had post-thrombotic syndrome.[3]

Alteplase is a recombinant tissue plasminogen activator, which initiates fibrinolysis by binding to fibrin in a thrombus surface and converts entrapped plasminogen to plasmin. Its specificity and short half-life are two advantages over the other available thrombolytics. In contrast, the antigenicity and low specificity of streptokinase limited its use in the management of thrombosis in pediatric patients. Urokinase was widely used in pediatric patients, but a US Food and Drug Administration warning has substantially diminished the use of urokinase in North America.[4] The efficacy and safety of alteplase in adults for management of thromboembolic events is well documented. Recent dose recommendations for alteplase thrombolysis in pediartrics were defined according to site of thrombus and age of the patient.[5] The available data for the management of thrombosis in neonates and children are predominantly from published case reports and no clear guidelines regarding alteplase use are available yet.[6-16] Therefore, we sought to retrospectively evaluate alteplase efficacy and safety in the management of intracardiac and major cardiac vessel thrombosis in pediatric patients.

METHODS

In this retrospective case series we evaluated neonates and children patients who developed intracardiac or major cardiac vessel thrombosis and in whom alteplase was used in the cardiac surgery intensive care unit (ICU) at King Faisal Specialist Hospital and Research Center, a tertiary care institution. All neonates and children, 14 years of age or younger, who used alteplase for the management of intracardiac or major cardiac vessel thrombosis from 1997 to 2004, were reviewed. The institutional Clinical Research Committee, as well as the Research Ethics Committee approved the study,

All patients who were less than 14 years old and received alteplase were identified through the pharmacy database at our institution. These patients were sorted by indication and only patients who used the medication for the treatment of intracardiac thrombus or major cardiac vessels were included in the study. Therefore, cases of pulmonary artery, femoral, descending aorta, or portal vein thrombosis and those diagnosed as endocarditis cases were excluded. Furthermore, patients had to have documented cardiac echocardiography done prior to and following alteplase administration. Patients also had to have symptoms of hemodynamic instability or persistent sepsis secondary to the presence of the clot indicating the need for thrombolysis.

Baseline characteristics including patient age, weight, gender, previous medical history, ICU admission period, use of a central venous catheter, total parenteral nutrition administration and whether surgery was done or not before the diagnosis of thrombus, were collected. A pediatric echocardiography consultant reviewed the initial echocardiogram and the baseline thrombus description, location, size, age (echogenic clot indicates an old clot whereas a non-echogenic clot indicates a fresh clot), and determined the degree of blood flow obstruction. Baseline coagulation profile, complete blood count, renal and hepatic profiles were also reviewed. The alteplase regimen including doses given, type of infusion (intermittent or continuous), duration and concurrent medications used were collected.

Post-therapy echocardiograms were reviewed for efficacy of alteplase therapy. Thrombus size and the presence or absence of blood flow and final assessment of dissolution at the time of alteplase discontinuation were assessed. A single echocardiogram specialist analyzed all images. At the time of evaluation, the echocardiographer was blinded to the timing of alteplase administration. Thrombus resolution was categorized as complete, partial, or negligible. Complete clot resolution was defined as the absence of clot as demonstrated echocardiographically on the last day of infusion. The clot was considered partially resolved if its size reduced or blood flow improved from baseline.[17] Fibrinogen and D-dimer levels used to monitor the response to alteplase were also collected when available.

Baseline and daily blood work collected during and 48 hours after the infusion included hemoglobin, hematocrit, activated partial thromboplastin time (aPTT), prothrombin time (PT), platelets, alanine aminotransferase (ALT), urea, and creatinine. Bleeding complications of the therapy were categorized into three categories: minor bleedings, major bleeding, and death. Bleeding at vascular puncture sites, oozing, or mucosal bleeding were considered minor events if no further sequelae were reported. Major bleeding events were those requiring blood product transfusion as a result of a hemoglobin level decrease of >4 g/dL after therapy, or gastrointestinal, or intracranial hemorrhage or bleeding from more than three sites after infusion.[18,19]

RESULTS

Nineteen pediatric patients were administered alteplase from 1997 to 2004. Six patients were given alteplase for the treatment of either intracardiac or major cardiac vessels thrombus, but only five had completed echocardiographic evaluation and thus met the study selection criteria. The sixth patient was treated with a single alteplase infusion, but he died shortly after and no further echocardiogram evaluation was done, and therefore he was excluded.

The baseline characteristics of the five patients are summarized in Table 1. Patient ages ranged from 40 days to 13 years and three were male. Only one case (Case B) had a genetic related homeopathy (Bahcet disease). A central venous catheter was placed before starting alteplase therapy in four patients (80%), for a median duration of 16 days (1 day up to 30 days). All five patients had compromised hemodynamics and four developed sepsis during their ICU stay. Three

Table 1. Baseline patient's characteristics.

Case	Age	Gender	Weight (kg)	ICU stay* (days)	Central venous catheter*	Patient history	Surgery	Sepsis
A	9 y	M	17	0	N	CHD, acute renal failure Bahcet disease, superior sagittal sinus	Fontan	Y
B	13 y	M	46	0	Y	Thrombosis, pulmonary embolism	Tricuspid valve replacement	N
C	40 d	F	3	42	Y	CHD (PDA, abnormal coronary arteries)	Arterial switch	Y
D	7 m	F	3.5	0	Y	Metabolic disease, chronic diarrhea of unknown cause, TPN dependent, impaired coagulation	None	Y
E	65 d	M	1.2	54	Y	CHD, VSD repair, PDA ligation	VSD and ASD closure	Y

*Before alteplase treatment, ASD: atrial septal defect, CHD: congenital heart disease, F: Female, M: Male, m: month; d: day, y: year, PDA: patent ductus arteriosis , TPN: total parenteral nutrition, VSD: ventricular septal defect

Table 2. Alteplase dosing regimens and concomitant antithrombotic agents for the five patients.

Case	Initial dose (mg/kg)	Type of infusion	Alteplase protocol	Total infusion time
A	0.5 (over 6h)	Intermittent	0.5 mg/kg/hr (6 hrs)	19 hrs
B	None	Intermittent	0.5 mg/kg/hr (6 hrs)	2 hrs
C	0.3 (over 6h)	Continuous	0.1 mg/kg/hr, decreased to 0.05 mg/kg/hr, then increased to 0.1 mg/kg/hr	96 hrs
D	0.5 (over 1h)	Continuous	0.2 mg/kg/hr, increased to 0.5 mg/kg/hr, then decreased to 0.2 mg/kg/hr, then increased to 0.5 mg/kg/hr	72 hrs
E	0.6 (over 1h)	Continuous	0.2 mg/kg/hr×24 hr, increased to 0.35 mg/kg/hr, then to 0.5 mg/kg/hr	48 hrs

patients were started on total parenteral nutrition for various periods. However, none of the study patients had any malignancy.

One patient was on anticoagulation therapy (warfarin in a dose of 2.5 mg daily) that was stopped upon commencing alteplase treatment (Case A), and another patient was receiving aspirin therapy (Case D). None of the five patients had a history of use of alteplase or any other thrombolytic. All patients received concomitant prophylactic heparin infusion according to the cardiovascular surgery intensive care unit protocol at our institution to achieve a target aPTT of 60-80 sec. The alteplase regimens are summarized in Table 2. The initial bolus of alteplase ranged from 0.3 mg/kg to 0.6 mg/kg followed by an infusion that ranged between 0.05 to 0.5 mg/kg/hr. Alteplase infusion time ranged from 2 hours to 96 hours.

The results of the echocardiography studies, efficacy and safety of the therapy in the identified five patients are summarized in Table 3. At baseline, echocardiography studies revealed total blood flow obstruction by the clot in two cases, moderate blood flow obstruction in two cases and no blood flow obstruction in one case. The two patients who received intermittent alteplase infusion and had "fresh" clots (Cases A and B) experienced complete clot lysis. The third patient with "fresh" clot (Case D) had a partial response as clot size was decreased, but the resolution of the blood flow obstruction was incomplete. Failure of treatment "negligible response" was documented in two patients; both had "old clots" at baseline and complete blood flow obstruction (Cases C and E).

All patients experienced bleeding events through different sites. Three patients (60%) had major bleeding events as previously defined (Cases C, D, E). All of the three cases received a continuous infusion of alteplase.

Table 3. Echocardiographic thrombus description at initial diagnosis and response to alteplase for the five patients.

Case	Site	Age of thrombus	Size (mm)	Severity of obstruction	Thrombus resolution	Safety outcomes
A	Fontan lateral tunnel	Fresh	2.5×3.7	Moderate	Complete	Minor bleeding
			1.7×2.3	Mild		
			None	None		
B	Anterior leaflet of the tricuspid valve	Fresh	1×0.5	None	Complete	Minor bleeding
			0.4×0.1	None		
			None	None		
C*	Left internal jugular vein	Old	Total occlusion	Total	Negligible	Major bleeding
			1×1	Moderate		
D	Superior vena cava	Fresh	0.8×0.9	Moderate	Partial	Major bleeding
			0.8×0.8	Mild		
E	Superior vena cava	Old	1.5×1.1	Total	Negligible	Major bleeding and death
			1.5×1.1	Subtotal		

*By Doppler ultrasound

Case C bled from three sites (endotracheal tube, chest tube and urinary tract) requiring multiple blood products transfusions. Case E bled profoundly through multiple injection sites (despite topical thrombin applications) and from the chest tube with a drop in hemoglobin of 2.5 g/dL, thus requiring multiple transfusions, and eventually died. Case D had severe melena with a drop of hemoglobin by 4.5 g/dL requiring multiple transfusions as well. The other two patients had minor bleeding events. There was oozing around chest tube and injection site in Case A and hemoptysis in Case B, although they were transfused at least once for reasons other than alteplase-related bleeding (blood products transfusion was commenced according to hemoglobin levels and platelets levels; to maintain hemoglobin level >100 g/dL, and platelets $>50\times10^3/\text{mm}^3$).

DISCUSSION

In our case series, the decision to start thrombolytic therapy was individualized with no standard protocol for alteplase use followed at our institution for the pediatric population. A median dose of 0.35 mg/kg/hr with a maximum infusion dose of 0.5 mg/kg/hr was associated with a 60% patency rate (2 complete, 1 partial lysis) achieved in an average duration of three days. The three cases had "fresh" clots; two were receiving intermittent dosing of alteplase and experienced no major bleeding complications. Hence, they required lower dosing, a shorter duration of therapy, and had a better outcome in comparison with the other two patients who had "old clots". The correlation of the clot age to thrombolysis efficacy needs further study; however, some investigators believe that for thrombolytic therapy to be effective the clot should be <3-5 days old and the presence of an organized clot (beyond 1-3 weeks of estimated age) may not respond to thrombolytic therapy.[20,21] If this is true, then we might need to critically screen our patients to identify those who are best candidates for thrombolytic therapy that would respond with the lowest effective dose and would have the lowest risk of hemorrhagic complications.

The recommendations for antithrombotic therapy including thrombolytics in children have been extrapolated from adult recommendations and are not well supported.[4,14] This is mainly due to the differences in the haemostatic system of children, making fibrinolytic therapy more difficult to manage. As such, there is no standardized dosing regimen for alteplase use in the neonatal population. Gupta and colleagues used a bolus of 0.1 mg/kg over 10 minutes followed by an infusion of 0.3 mg/kg/hr for 8 hours with good efficacy and safety, while Hartman's protocol used an initial bolus of 0.7 mg/kg over 30-60 minutes followed by a continuous infusion of 0.2 mg/kg/hr leading to a 94% patency rate without major bleeding complications.[13,14] Higher doses have been used effectively with no complications, including a case report by Mathur and colleagues where alteplase was used in escalating doses up to 0.25 mg/kg/hr for 6 hours for 9 days leading to the dissolution of a large organized intra-atrial cathether tip thrombus.[22] The doses of alteplase used in our case series are within the dose range used in previously reported cases in the management of pediatric thrombosis (0.01-1 mg/kg/hr with or without bolus dose).[6-16] Moreover, as in our cases, the outcome in these cases varied from no lysis of the clot to partial or complete resolution. When encountered, bleeding ranged from minor as from puncture sites to major intracranial hemorrhage. Two of our patients had minor bleeding, while three had major bleeding complications. Both patients who developed major bleeding received alteplase for a prolonged period of time (48-72 hours). Previous cases of the use of alteplase in the neonates and children have used alteplase for up to 240 hours, but most used alteplase for less than 24 hours.[6-16] The incidence of bleeding in these cases varied from minor to major (up to 56%), but no definitive correlation between the duration of the infusion and the incidence of hemorrhagic complications can be concluded. Furthermore, two of the major bleeding cases were in neonates and one was a preterm. Previous reports have suggested that preterm patients, due to their prematurity, are at an increased risk of bleeding.[23] This could be another reason for these patients to receive lower doses and a shorter duration of therapy; however, further studies are needed to confirm this finding.

In our series, fibrinogen levels were documented in four patients after the initiation of alteplase and we could not identify a direct correlation between these levels and the time of infusion; however, all of the results were above the recommended levels. It is well known that the fibrinogen level and its degradation products are useful safety and efficacy monitoring parameters, and can guide alteplase infusion adjustment if followed regularly. A fibrinogen level was recommended by Zenz et al, Gupta et al, and required in Levy's protocol.[7,10,14] Fibrinogen levels need to be maintained above 100 mg/dL and measured before initiation of alteplase and 4 hours later or when there is dose adjustment. However, adequate monitoring of degradation products and fibrinogen levels does not insure that the patient will not experience bleeding complications. Mueller and colleagues showed that there is no statistically significant correlation between bleeding complications and fibrinogen levels in patients with myocardial infarction who were receiving alteplase.[24]

Our results should be interpreted with caution because of the retrospective nature of the study in addition to the lack of complete laboratory data to evaluate the influence of alteplase on parameters like fibrinogen and D-dimers that can be an excellent monitor of bleeding outcomes. The small sample size and the wide age variation of our subjects are two other limitations of our study.

In conclusion, alteplase in an initial dose of 0.3 up to 0.6 mg/kg along with heparin therapy and followed by continuous, intermittent infusion may effectively dissolve intracardiac thrombi in the pediatric population especially when the thrombus is fresh. A longer duration of therapy was associated with an increased risk of major bleeding. Optimal dose, duration of infusion and clear monitoring guidelines still need to be identified. Further prospective studies with a large sample size evaluating the efficacy and safety of alteplase management of cardiac-related thromboembolic events in pediatric patients are warranted.

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Fetal valproate syndrome in a 2-month-old male infant

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Fetal valproate syndrome (FVS) results from prenatal exposure to valproic acid. It is characterized by a distinctive facial appearance, a cluster of minor and major anomalies and central nervous system dysfunction. We describe a 2-month-old male infant with the typical dysmorphic features characteristic of FVS. He had a persistent left superior vena cava draining into a dilated coronary sinus and mild pulmonary hypertension. There was a history of maternal intake of sodium valproate during pregnancy.

Valproic acid (VPA) is a widely used antiepileptic drug and mood stabilizer. It was first introduced for use as an antiepileptic drug in 1964 and is still a commonly used antiepileptic drug (AED) worldwide.[1] A description of the teratogenic effect of the drug was first published in 1980.[1] Since then many potential teratogenic and dysmorphogenic effects of VPA have been reported. We report a case of fetal valproate syndrome (FVS) in a 2-month-old male infant born of an epileptic mother who was taking sodium valproate during pregnancy.

CASE

A 2-month-old male infant was brought to our outpatient department for evaluation of bilateral inguinal hernia. He was the first child born of a non-consanguineous marriage. His mother had been taking sodium valproate for epilepsy for the past 12 years and was on a dose of 1200 mg/day throughout her pregnancy. The infant was born at full term at a peripheral hospital by emergency by cesarean delivery, the indication being fetal distress. His birth weight was 2 kg. The baby cried immediately after birth. He was admitted to the NICU for respiratory distress and was discharged on breast feeds on the third postnatal day. The intrauterine growth retardation and the teratogenic effects of the antiepileptic drug were not considered by the health care providers at the peripheral hospital. Hence, no specific tests were done in the antenatal period.

On examination at our hospital, his weight was 1.5 kg and length 52 cm (below 5th centile for age and sex) suggestive of failure to thrive. On enquiry the mother gave a history of nasal regurgitation of milk since birth. Examination of the oral cavity revealed the presence of a cleft in the soft palate (Figure 1). Dysmorphic features were present in the form of low-set ears, a broad and depressed nasal bridge, long philtrum, upturned nose, thin upper lip, thick lower lip, thin vermillion borders, small mouth, and medial deficiency of eyebrows (Figure 2). Broad hands and feet, bilateral radial club hand, loose skin, bilateral inguinal hernia (Figure 3) were other features.

Two-dimensional echocardiography showed a persistent left superior vena cava draining into a dilated coronary sinus and mild pulmonary hypertension. Ultrasound of the abdomen showed no abnormality. With a history of maternal intake of sodium valproate during pregnancy and typical dysmorphic features, a diagnosis of fetal valproate syndrome (FVS) was made.

DISCUSSION

None of the AEDs available currently are completely safe during pregnancy, but VPA appears to be the most teratogenic. In a study done by Morrow et al on the major congenital malformation (MCM) risks of antiepileptic drugs in pregnancy, the overall risk for all AED exposed cases was 4.2%. The MCM rate was higher for polytherapy than for monotherapy. Polytherapy regimens containing valproate had significantly more MCMs than those not containing valproate. For mono-

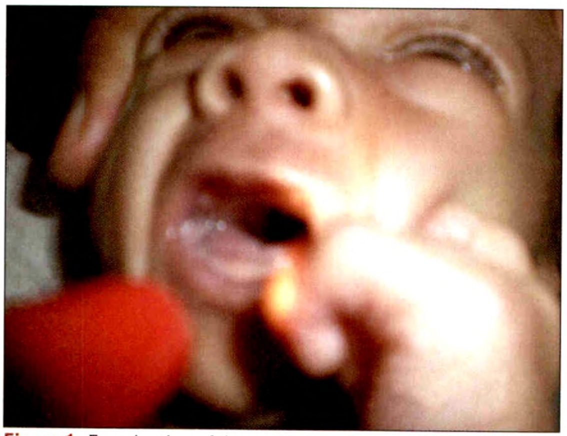

Figure 1. Examination of the oral cavity showing isolated cleft of the secondary palate.

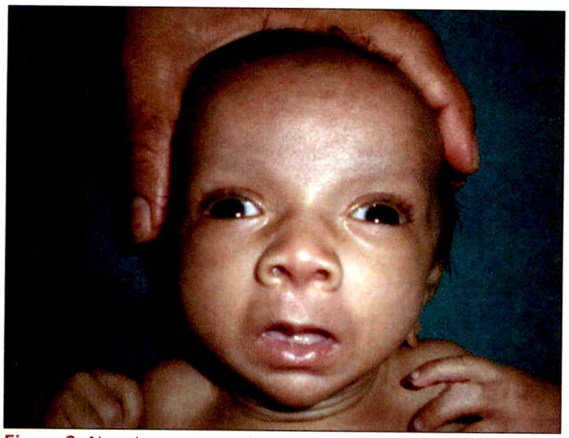

Figure 2. Note low set ears, broad and depressed nasal bridge, long philtrum, upturned nose, thin upper lip, thick lower lip, thin vermillion border, small mouth, and medial deficiency of eyebrows.

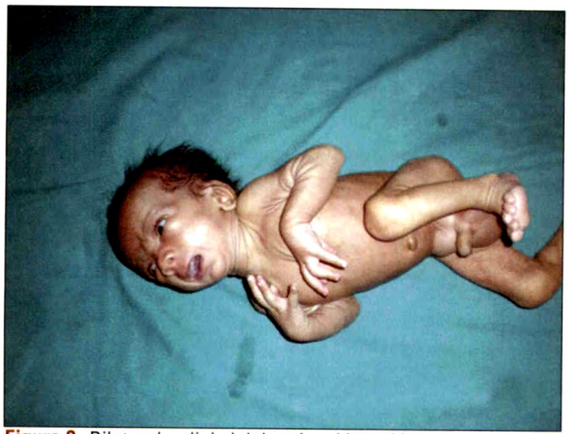

Figure 3. Bilateral radial club hand and broad hands, loose skin, bilateral inguinal hernia.

therapy exposures, carbamazepine was associated with the lowest risk of MCM and those exposed to more than 1000 mg of valproate had the highest MCM rate than for any other monotherapy exposure.[2]

There is also a controversy as to whether epilepsy itself contributes to antiepileptic drug teratogenecity, thus confounding the teratogenic risk of a drug used for its treatment. However VPA, and not the underlying epilepsy syndromes, has been found to be associated with the elevated risk for malformations in the drug exposed fetus.[3] VPA is associated with the highest risk of birth defects, especially with doses exceeding 1000 mg/day.[4] Various factors contribute to the teratogenecity of VPA. These include the number of antiepileptic drugs that are co-administered, drug dosage, differences in maternal and/or infant metabolism and the gestational age of the fetus at exposure.[5] VPA crosses the placenta and is present in a higher concentration in the fetus than in the mother. There is a 6 to 7 times increased risk of congenital malformations in babies of mothers exposed to valproate. Major congenital malformations are neural tube defects, congenital heart defects, oral clefts, genital abnormalities and limb defects. Other less frequent abnormalities include inguinal and umblical hernia, supernumerary nipple, postaxial polydactyly, bifid ribs and pre-axial defects of the feet.[6] Zinc deficiency has been suggested as a possible cause of neural tube defects associated with valproate exposure as sodium valproate readily binds to zinc.[7] Our patient had the typical facial features, bilateral inguinal hernia, low birth weight, upper limb and cardiac malformation. A distinctive facial phenotype of fetal valproate syndrome has been described which tends to evolve with age. The facial features are tall forehead with bifrontal narrowing, medial deficiency of eyebrows, infraorbital groove, trigonocephaly, flat nasal bridge, broad nasal root, antiverted nares, shallow philtrum, epicanthic folds, long upper lip with thin vermillion borders, thick lower lip and small downturned mouth.[5]

There is a 10 times increased risk of neural tube defects in babies of mothers exposed to VPA. The risk is maximum with VPA therapy, as compared to other anticonvulsants. The incidence of congenital heart disease is estimated to be around 4 times than that seen in the general population.[5] Aortic valve stenosis, secundum atrial septal defect, pulmonary atresia without ventricular septal defect, perimembranous ventricular septal defect, interrupted aortic arch, hypoplastic left heart syndrome septal defects and valvular problems are known to occur.[6] Our case had left superior vena cava opening into a dilated coronary sinus.

Oral clefts are 5 times more frequent than expected.

Limb defects, digital abnormalities of various types and minor skin defects are known to occur.[5] The association of developmental delay with VPA exposure has also been described.[5] Pregnancy usually proceeds uneventfully and 10% of babies are small-for-gestational age as in our patient. Postnatal growth appears to be normal and general health is good.[3] Microcephaly tends to occur only in the infants who are also exposed to other anticonvulsants. Prenatal diagnosis is focused on the detection of neural tube defects (NTD), as they are the proven major malformations. Estimation of maternal serum alpha-fetoprotein (AFP) can be used as a screening test for the presence of open NTD.

During pregnancy antiepileptic drugs should be administered as monotherapy and in the lowest possible dose with constant monitoring of serum concentration of antiepileptic drugs.[8] VPA should not be routinely prescribed to women of child-bearing potential. If there is no effective alternative, then doses should be limited to a maximum of 1 gram per day, administered in divided doses and in the slow release form. Also high dose folic acid (5 mg/day) is recommended during pregnancy, starting at least 6 weeks pre-conception and continuing through the first trimester.[8]

The efficacy of VPA as an AED cannot be disputed, but the extent of its teratogenic effects cannot be under-estimated either. Hence, a balance between the therapeutic effects of this drug and its teratogenic effects is critical in the management of pregnant women with epilepsy.

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Synchronous double primary lung cancers via p53 pathway induced by heavy smoking

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Differences in the histological manifestation of synchronous lung cancers are rare. Synchronous multiple primary lung cancers (SMPLC) are associated with long-term tobacco use, which could independently lead to mutations in the p53 and K-ras genes. We present the case of an 82-year-old man who smoked 30 cigarettes daily for the past 60 years. CT of the chest showed a right upper lobe mass. Bronchoscopy revealed an intra-lumen nodular lesion in the right lower lobe bronchus. The diagnoses of small cell lung carcinoma (SCLC) of the right upper lobe and non-small cell lung carcinoma (NSCLC) of the right lower lobe were confirmed by the morphologic features and the detected immunoreactivity. Immunohistochemical analyses showed a strong positive reaction for p53 in samples of the SCLC and NSCLC. The cancers had a different phenotype, but similar genetic abnormalities may have developed due to the carcinogens in the cigarettes.

It is difficult to distinguish multiple synchronous lung tumors from multicentric lung cancers, or from a single lung cancer with intrapulmonary metastases or pulmonary metastases that have originated from primary cancers in different organs. These cancers may be distinguished as a second primary lung cancer on the basis of different molecular genetic characteristics or in the absence of radiologic features of mediastinal node involvement.[1] A synchronous second focus of lung cancer in a different lobe is easily defined as a second primary lung cancer when the two sites of tissues are different histologic types. Clinical trials have shown that the incidence of synchronous multiple primary lung cancers (SMPLC) ranges from 1% to 7%.[2,3] The occurrence of synchronous lung cancers, which is defined by a second tumor in a different lobe and with a different histological manifestation, is rare.[3-5] In 1953, Slaughter et al proposed that those preneoplastic and neoplastic lesions are usually multifocal and develop throughout the entire respiratory tract because of exposure to similar carcinogens. They referred to this phenomenon as the concept of "field cancerization".[6] Sozzi et al supported this hypothesis and showed that cytogenetic abnormalities could be detected in epithelial cells of these tumors and that these cytogenetic changes occur in cells distant from normal appearing epithelial cells.[7]

CASE

An 82-year-old male was admitted to our hospital because of cough with blood-streaked phlegm. These symptoms had been sustained for two weeks. He had smoked 30 cigarettes per day for the past 60 years. The patient did not experience dyspnea, fever, chest pain, body weight loss, or poor appetite. His medical history and family history were unremarkable. Physical examination on admission revealed a clear breathing sound; moreover, abnormalities in neck size as well as supraclavicular lymph nodes were not detected. The results of the laboratory tests were within the normal range except for a mild normocytic anemia.

On performing routine chest radiography, a mass lesion on the right upper lobe was discovered (Figure 1a). Contrast-enhanced CT revealed a lobulated soft-tissue mass that measured 3.7 cm in the largest dimension on the axial plane in the medial region of the right upper lobe (RUL). In addition, the image showed encasement of the RUL bronchus, mediastinal invasion, and enlarged lymph nodes in the pretracheal retrocaval space, subcarinal space, and the right hilar region (Figure 1b). Bronchoscopic examination revealed extensive submucosal and lymphangitic infiltration with partial obstruction of the right upper lobe orifice and a whitish keratinized tumor, which bled easily upon touch, and partially occluded the right anterior segment of the lower lobe bronchial lumen (RB7, right anterior segment of the lower lobe bronchial lumen) (Figures 1c, 1d).

Pathologic examination of the pulmonary specimen of the RUL bronchus revealed small cell lung cancer (SCLC), which was positive for chromogranin-A and negative for CD45. The pulmonary specimen of the bronchus of RB7 showed moderately to poorly differentiated non-small cell lung carcinoma (NSCLC). In addition, the morphological features and immunohistochemical results of the tumor cells from the two distinct regions of the right lung were different (Figure 2). These results were not consistent with the characteristics reported for metastatic cancer of pulmonary origin. However, both pathologic specimens showed a strong positive reaction for p53, implying that similar carcinogenesis caused these two different types of lung cancers. We also performed an abdominal sonography and a Tc-99m whole body bone scan. The findings were unremarkable. The patient refused further examinations, was discharged and was not available for a follow-up visit.

DISCUSSION

The occurrence of SMPLC has been reported to be approximately 1%.[8] Small differences with regard to the etiology and clinical manifestation of SMPLC are difficult to detect due to the few number of cases; hence, there have been no reports on a definite etiology and clinical manifestations of SMPLC. In 1999, Wang et al demonstrated that patients with SMPLC had significantly more tobacco exposure. In addition, they reported that these tumors developed independently due to mutations in the p53 and K-ras genes, which suggested that field cancerization may be an important aspect of lung carcinogenesis.[9]

The etiology of lung cancer is strongly associated with cigarette smoking. Exposure to carcinogens found in tobacco causes various genetic alterations in the genome of susceptible pulmonary cells, indicating molecular tumorigenesis during multistep tumor progression.[10] The most frequent genetic abnormalities occur in tumor suppressor genes (TSGs). The TSG p53 regulates both cell cycle progression and apoptosis. It plays a key role in protecting cells from duplicating damaged

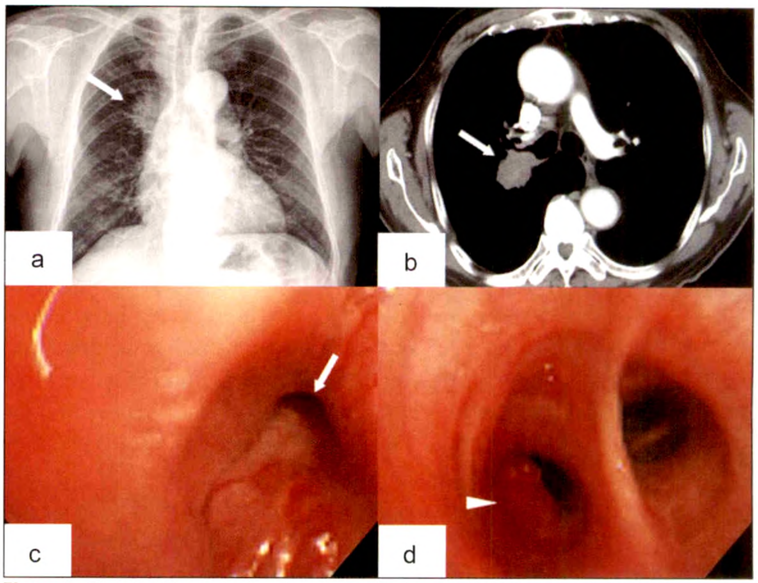

Figure 1. Chest radiograph showing a mass lesion on the right upper lobe (a). The contrast-enhanced CT of the chest shows a right upper lobe soft-tissue mass with encasement of the bronchus, mediastinum invasion and an enlarged right hilar lymph node (b). The bronchoscopic image shows extensive submucosal and lymphangitic infiltration with partial obstruction of the right upper lobe orifice (c) and one whitish, keratinized tumor, easy to bleed on touch and partially occluding the RB7 bronchus lumen (d).

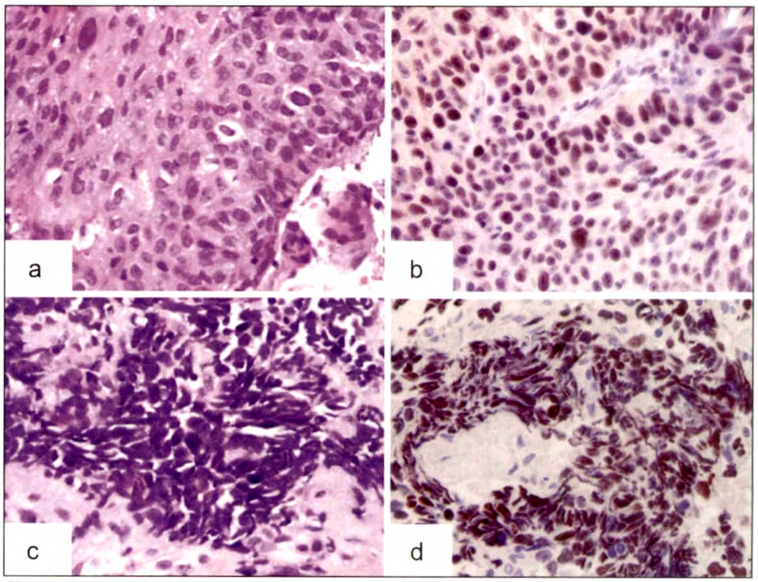

Figure 2. Sections of the right upper lobe lung lesion showing small cell carcinoma characterized by small, pleomorphic tumor cells with nuclear molding and "crush" artifacts (a,×400). The tumor cells were immunohistochemically positive for p53 (b,×400). The images of sections of the RB7 lung lesion show non-small cell lung carcinoma with pale to pink cytoplasm without specific differentiation (c,×400). The tumor cells were immunohistochemically positive for p53 (d,×400).

DNA. Increased expression of mutant p53 induces failure of apoptosis. This is accompanied by uncontrolled cell proliferation leading to neoplastic transformation. The TSG p53 is mutated in more than 90% of SCLCs and more than 50% of NSCLCs. In our case, specimens of SCLC and NSCLC were immunohistochemically positive for p53, suggesting that although their phenotypes are different, a similar genetic abnormality caused by carcinogens of cigarette smoking may have led to the development of SCLC and NSCLC. A recent study analyzed 70 lung tumors from 30 patients with concordant genetic alteration examination, and concluded that the majority of multifocal lung cancers had a common clonal origin. This finding may support the presentation of SMPLC in our case.[11]

Multiple synchronous tumors of histologically different cell types should be considered as separate primary lung cancers and should be staged separately.[12] Mediastinoscopy and systemic staging are recommended in order to exclude the possibility that one pulmonary lesion metastasized from the other and that both lesions represent systemic metastases originating from a different tissue. The 5-year survival rate of patients diagnosed with SMPLC has been reported to be significantly lower than in patients with single primary tumors.[13] The poorer prognosis for multiple cancers may be explained by the different biological features. In addition, some studies suggest that surgery should not be performed if the tumors have advanced beyond stage II.[3,14] However, Chang et al proposed that multiple primary lung cancers could be regarded as a local disease rather than a systemic disease, and that they are potentially curable, especially with no metastatic lymph nodes.[15] In 2007, Trousse also suggested that patients with SMPLC are expected to benefit from surgery after an appropriate selection process (node-negative patients with adequate pulmonary reserve).[16] In patients with multiple synchronous lesions, limited surgical procedures to remove the synchronous lesions are preferred, especially since pneumonectomy has been associated with an increased risk of death.[16]

In conclusion, SMPLC is a rare clinical entity and the mechanism of carcinogenesis is still unknown. Heavy smoking resulting in carcinogenesis via the induction of the p53 mutation may play a role in SMPLC. Therefore, studies with large sample sizes are warranted.

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case report

Delayed presentation of severe combined immunodeficiency due to prolonged maternal T cell engraftment

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Severe combined immunodeficiency (SCID) is a primary immunodeficiency disorder with heterogenous genetic etiologies. We describe a typical case in a 9-year-old boy that was masked by a clinically functional maternal T cell engraftment leading to late presentation with *Pneumocystis jiroveci* pneumonia and cytomegalovirus infection, probably following exhaustion of maternally engrafted cells. Based on immunological findings, he had a T- B+SCID phenotype.This report suggests that in rare cases, engrafted maternal T cell might persist for long time leading to partial constitution of immune function and delayed clinical presentation of SCID.

Severe combined immunodeficiency (SCID) is a primary immunodeficiency disorder with heterogeneous genetic etiologies, characterized by a profound defect in both T and B lymphocytes.[1,2] Affected individuals usually present in early infancy with severe and persistent infections.[3] Without hematopoietic stem cell transplantation (HSCT) or gene therapy, most patients die in early childhood.[4,5] Transplacentally derived maternal T lymphocytes are frequently detected in healthy newborns; however, they are rapidly eliminated by immune competent T lymphocytes.[6,7] On the contrary, SCID infants do not usually reject maternally engrafted cells; therefore maternal T cells were detected in 24% to 40% of patients undergoing hematopoietic stem cell transplantation.[8,9] Since these T cells are usually non-functional, they do not alter the course of the disease and patients present typically in early infancy with severe infection.[9] We present a typical case of SCID masked by a clinically functional maternal T-cell engraftment leading to late presentation of the disease at the age of 9 years with *Pneumocystis jiroveci* pneumonia (PJP) and cytomegalovirus (CMV) infections, probably following exhaustion of maternally engrafted T lymphocytes.

CASE

A 9-year-old Saudi Arabian boy was referred to our hospital for further investigation of slowly resolving pneumonia. He was a product of full-term, uneventful pregnancy, with good birth weight and had an unremarkable neonatal period. Apparently, he remained healthy until the age of 5 years when he started to have recurrent attacks of cough and dyspnea, which were treated with bronchodilator and prophylactic steroid in addition to frequent use of oral antibiotics, with good response. Six months prior to presentation to our hospital, he started to show gradual clinical deterioration. He presented with lower respiratory tract infection not responding to several courses of oral antibiotics. He had no history of recurrent otitis media or sinusitis, no history of skin abscesses, dermatitis or any other skin lesions, and no history of chronic diarrhea. He received all vaccinations as per routine schedule with no apparent complications. The parents were first-degree cousins, but apart from the atopy which both parents have, there was no history of immune deficiency, chronic lung disease, recurrent infections, or early deaths.

On physical examination, he showed signs of respiratory distress, tachypnea and hypoxia. His height

(120 cm) and weight (17 kg) were below the 3rd centile with weight far more affected than height. There were no dysmorphic features, but he had grade three clubbing of the hands and feet. The tonsils were normal and there was no lymphadenopathy. Although he had received BCG vaccine at birth, there was no evidence of BCG scar. On chest examination, there was coarse crepitation bilaterally. Other systemic examinations were unremarkable. The patient was put on broad spectrum antibiotics. CT scan of the chest showed bronchiectatic changes. Sweat chloride test was normal at two different time points (30 and 35 mmol).

Table 1. Immunological findings.

	Patient value	Normal age-matched value
Immuoglobulin		
IgG (g/L)	2.35	6-15.7
IgA (g/L)	0.49	0.45-2.3
IgM (g/L)	0.56	0.52-2.4
IgE (IU/L)	3022	<350
Antibody response (titre)		
Anti-tetanus toxoid IgG IU/mL	0.09	
Anti-tetanus toxoid IgG 1 mg/L	0.7	
Anti-pneumococcal IgG IU/mL	<3.30	
Anti-pneumococcal IgG 2 mg/L	<1.11	
Lymphocyte subpopulation ($\times 10^9$/L)		
CD2	1600	2000-5000
CD3 (%)	943 (66%)	1700-1900
CD4 (%)	380 (28%)	800-1700
CD8 (%)	417 (30%)	700-1000
CD19 (%)	424 (28%)	400-800
CD16-56 (%)	57 (4%)	200-400

Table 2. In vitro proliferation assays.

Mitogen	Patient CPM^a (%)	Control 1 (CPM^a)	Control 2 (CPM^a)
PHA	9518 (7%)	154 571	1 141 534
Con A	8311 (10%)	86 589	76 009
PWM	20 976 (37%)	66 017	51 439
Pooled allogeneic cells	3023 (4%)	44 054	40 543

^{a}CPM: count per minute, PHA: phytohemagglutinin, ConA: concanavalin A, PWM: pokeweed nitrogen

Immunological findings are shown in Tables 1 and 2. The high IgE level indicated normal isotype switching and thus ruled out the hyper IgM syndrome. This possibility was further excluded by the intact expression of CD40 on the surface of B lymphocytes and CD154 on CD4 T cells post PMA stimulation for 4 hours. We were not able to test the ability of B cells to mount antibody response as he was started on intravenous immunoglobulins; however, they appeared to produce reasonable pre-vaccination levels. HIV tests for both antibodies and RNA levels were negative. The adenosine deaminase B level was 1.5 IU/g Hb (normal range, 0.3-1.5 IU/g Hb). A purine nucleoside phosphorylase deficiency was unlikely with normal uric acid level (171 µmol/L; normal range, 60-240). Short tandem repeat analysis of patient peripheral blood showed 2.4% maternal T lymphocytes engraftment and 2.6% myeloid cells engraftment. The HLA typing showed full compatibility with his mother. Lung biopsy revealed eosinophilic infiltrate and a Grema stain was positive for PJP (Figure 1). Based on these data, he clearly had a T- B+ SCID phenotype with maternal T cell engraftment. Therefore, he was treated with intravenous co-trimoxazole (Septrin, GlaxoSmithKline, UK) for four weeks with a good clinical response and placed on intravenous immunoglobulin replacement therapy. HSCT was initiated. Subsequently, he was admitted again with a CMV infection confirmed by a high CMV viral load that responded well to ganciclovir. The patient was screened for mutation in *RAG1*, *RAG2* and Artemis genes via genotyping and direct gene sequencing, but no mutation was detected, possibly because the mutations were outside the coding regions of the screened genes, or possibly there were defects in other candidate genes, some of which might be novel, that are yet to be discovered in this patient. He was being investigated to delineate the underlying genetic defect.

The HLA typing was done on peripheral blood lymphocytes and on epithelial cells from a buccal smear. HLA class I low resolution and HLA Class II high resolution typing was performed. HLA typing was performed using sequence-specific oligonucleotides (Lifematch, Tepnel Lifecodes Molecular Diagnostics, Stamford, CT 06902, USA) and sequence specific primers (Invitrogen Corporation, Carlsbad, CA 92008, USA) described elsewhere. It revealed full matching between the patient and his mother as follows: A*02, A*26, B*49, B*50, Cw*06, Cw*07, DRB1*0701, DRB1*1301, DRB3*01/02/03, DRB4*01, DQB1*0202, DQB1*0603; and for the father: A*26, A*68, B*50, B*51, Cw*06, Cw*15

DISCUSSION

It is well documented that maternal cells are able to cross the placenta and be detected in the healthy newborn.[6,7] In an immune competent host, these maternal cells are usually rejected by the host T cell lymphocytes, but they live longer in infants with impaired cellular immunity as SCID. These cells are usually malfunctional and are not able to change the course of the disease due to their impaired or absent in vitro proliferative responses to mitogens.[7-9] However, a recent report of an 8-year-old child with SCID who presented with modest recurrent infections and detectable maternal cells in the peripheral circulation indicated that such cells may provide the required immunocompetence and result in prolonged survival in patients with SCID.[10]

Although our patient presented with recurrent chest infection leading to bronchectasis, he survived until the age of 9 years, probably due to maternal T and myeloid cells engraftment enhanced by HLA compatibility with his mother. These cells are believed to play an important role in the T, B and antigen-presenting cell cooperation in view of parental consanguinity, which has been found in 56% of Saudi population.[11] The maternal T cells in our patient were detected in a low percentage (2.4%), which probably has declined over time with apparent exhaustion of their function and inability to provide efficient immune constitution, evidenced by the significantly reduced T lymphocyte proliferation response to mitogens and predisposition to opportunistic infections as PJP and CMV.

Maternal T cells in SCID infants have the capacity to mediate undesired immune function despite their inability to respond efficiently to mitogens in vitro manifesting by allograft rejection and immune cytopenia, in addition to their ability to cause GVHD.[12,13] In a large cohort of 121 infants with SCID undergoing HSCT, maternal T cells persisted in 48 (40%) of the patients.[8] The majority were asymptomatic, while 19 (40%) of the 48 patients had findings consistent with GvHD, mainly involving the skin.[8] It was shown that the low level of maternal T lymphocytes associated with low incidence of GvHD, which could explain the lack of GvHD symptoms at the time of presentation in our patient; as maternal T cells were found only at 2.4%. Our patient underwent HLA-matched unrelated cord blood stem cell transplantation. Although he engrafted initially with mixed chimerism, he eventually lost his graft (Figure 2). It has been shown that transplacentally acquired maternal T cells might cause allograft rejection and immune cytopenia, which probably explain the allograft rejection in our patient.[12]

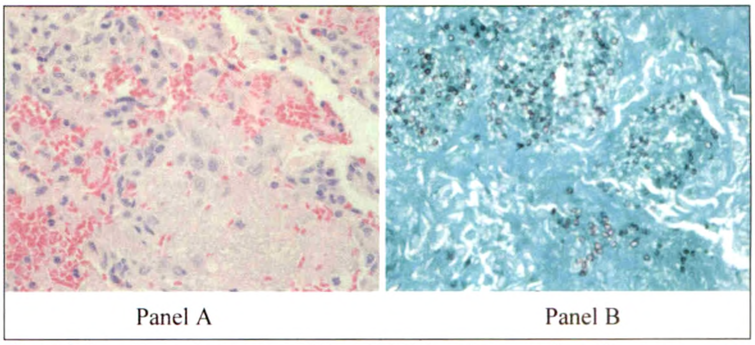
Panel A
Panel B

Figure 1. Lung biopsy from 8-year-old boy with delayed presentation of severe combined Immunodeficiency and maternal T cell engraftment. Panel A shows mild alveolar wall thickening and predominant intra-alveolar eosinophilic infiltrate; panel B shows special stain for a Giemsa stain with heavy growth of *Pneumocystis jiroveci* pneumonia.

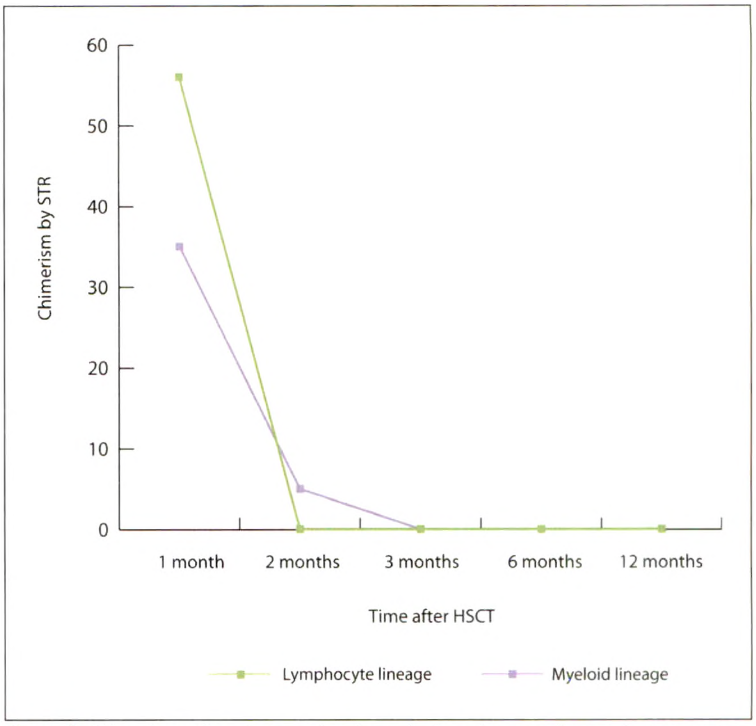

Figure 2. Chimeric studies after unrelated matched cord stem cell transplantation over 12 months period showing early but non-sustained engraftment. The allograft rejection is probably caused by maternal T cells. STR: short tandem repeat

In conclusion, transplacental maternal T cell engraftment may provide prolonged, but not persistent immunity in SCID in rare cases, and it should be suspected in late presentation of SCID. These cells usually exhaust overtime and patients should go for long-term intervention either by HSCT or gene therapy.

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erratum

In Volume 30, Issue 2, in the following article: Al-Mahdi M, Al Mutair A, Al Balwi M, Hussain K. Successful transfer from insulin to oral sulphonylurea in a 3-year-old girl with a mutation in the *KCNJ11* gene. Ann Saudi Med 2010;30:162-164

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Oral contraceptives alter oral health

To the Editor: The past 50 years have dramatically improved perceptions concerning the actions of sex steroid hormones in health and disease. Basic, clinical, and epidemiological research has not only increased our knowledge of the role of sex steroid hormones in reproductive endocrinology, but has also improved awareness of chronic diseases such as osteoporosis, cardiovascular diseases, cognitive disorders, and periodontal diseases that affect women's health.[1] In general, women with pre-existing gingival conditions or susceptibility to periodontal disease may experience an exacerbated response to bacterial plaque if they are pregnant, use oral contraceptives, have hormonal replacement therapy, or undergo menopause. Women on hormonal replacement therapy and oral contraceptives experience a statistically significant increase in gingival inflammation.[2,3] With oral contraceptives, this increase in gingival inflammation is related to the duration of use, and results of recent studies suggest that prolonged use of oral contraceptives may detrimentally affect the periodontium.[4] In the gingiva, numerous clinical studies have characterized sex hormone-induced changes in phenotype, preferential accumulation, and metabolization of estrogen and progesterone, as well as the presence of estrogen and progesterone receptors.[1] Periodontitis is one of the most ubiquitous diseases[5] and periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession, or both.[6] Research has demonstrated that the host response to periodontal infection results in the local production of cytokines and biological mediators such as prostaglandins and interleukins, as well as the systemic production of serum antibodies.[5] Women taking oral contraceptives demonstrate a significant increase in the number of *Prevotella* species in the gingival microflora. Increased female sex hormones substituting for the naphthoquinones required by certain *Prevotella* species most likely are responsible for this rise, and measurable changes have also been observed in the salivary components of women taking sex hormones, including a decrease in concentrations of protein, sialic acid, hexosamine fucose, hydrogen, and total electrolytes; in some studies, changes in salivary flow have been reported.[7] Women using contraceptives had 16-fold higher levels of *Bacteroides* species[5] and experience a 2-fold to 3-fold increase in the incidence of localized osteitis following extraction of mandibular third molars.[8] Thus, because of the effects of hormonal changes and their corresponding impact on the periodontium, proper periodontal evaluation and treatment for these women is recommended. The negative influence of the changes in estrogen and progesterone levels can be controlled by additional plaque control. Current users of oral contraceptives had poorer periodontal health[9] so dental examination should be an integral part to be followed up strictly and regularly to avoid any future dental complications. Thus, the emergence of periodontal medicine demands that dentists assume a larger accountability for the overall health of their patients, and acquire information of relevant systemic conditions to interrelate more meaningfully with medical colleagues to accomplish the final goal of providing superior patient care.

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Granulomatous gall bladder: A surgico-pathological challenge

To the Editor: *Mycobacterium tuberculosis* can affect any part of the gastrointestinal tract from the mouth to the anal canal along with the other organs of the peritoneal cavity, but the gall bladder is unlikely to be primarily involved. The incidence of localization of the tubercular bacilli in the gall bladder is uncommon as the intact gall bladder mucosa is resistant to the bacterium because of the concentrated bile acids present in the organ. The spread of infection to the gall bladder could be hematogenous, from an adjacent caseating lymph node or from the peritoneum.[1] Cholilithiasis or cystic duct obstructions are thought to be essential for the development of gall bladder tuberculosis.[1,2]

A 26-year-old female presented to the surgical outpatient department with right upper on-and-off abdominal and epigastric pain with dyspepsia for 8 months. After taking a detailed clinical history, ultrasonography of the abdomen revealed a thickened gall bladder wall with multiple gall stones, leading to a diagnosis of chronic cholecystitis with cholelithiasis. Gastroduodenoscopy of the patient was normal. X-ray of the chest and all other hematological parameters were also normal. The patient underwent a routine laparoscopic cholecytectomy, which was uneventful. After the extraction of the gall bladder, the specimen was opened to reveal a palpable nodular induration in the wall at the fundus of the organ. As facilities for frozen section biopsy were unavailable, and the possibility of malignancy was present, an imprint cytology was sent, which demonstrated abundant epithelioid-like cells with some necrosis. No malignant cells were seen. The surgery was terminated and the histopathology closely followed. Tuberculosis of the gall bladder showing caseous necrosis with Langhans' giant cells with epithioloid cells and lymphocytes (Figure 1) was diagnosed. Polymerase chain reaction (PCR) for tuberculosis confirmed the diagnosis. The patient was put on standard antitubercular chemotherapy for 9 months, after which she had regular follow-ups and was asymptomatic at 11 months after surgery.

The first case of tuberculosis of the gall bladder was described in 1870 by Gaucher.[3] Gastrointestinal tuberculosis usually presents as involvement of the peritoneum, intestine, or the lymph nodes, and isolated tuberculosis of the gall bladder is extremely rare with only about 50 cases reported in literature.[4] In a study of 280 patients with hepatobiliary tuberculosis, only one case had gall bladder involvement.[5] In their experience over a period of two decades with gall bladder and pancreatic tuberculosis, Saluja et al[6] found only three cases of the gall bladder being involved with the tuberculous infection. The rarity of the disease involving the gall bladder could be attributed to the fact that bile is thought to have an inhibitory effect on the gall bladder mucosa for the development of tuberculosis. Cholelithiasis and cystic duct obstruction are considered important factors for its development,[1,2] as seen in our patient. There are very few reports in literature of acalculous cholecystitis with tuberculosis.[7]

There are no pathognomic features for the preoperative diagnosis of gall bladder tuberculosis[3] and accidental reporting on histopathology has been a common occurrence.[2] This case is reported to emphasize the opening of the gall bladder in the operating room after its extraction from the peritoneal cavity because of the suspicion of any intraluminal lesions, and thus to alert the reader of this rare pathology.

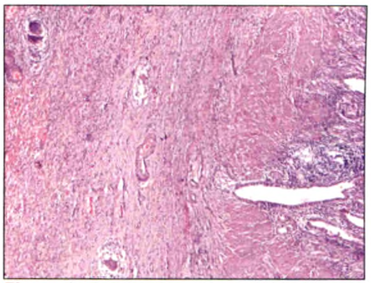

Figure 1. Section from the gall bladder showing multiple epithelioid granulomas and Langhans giant cells in the serosa with lymphocytic infiltration in all the layers (hematoxylin and eosin, ×50).

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RE: Reactive macrophage activation syndrome (MAS) in a patient with parvovirus B19 infection, lymphocytic lichenoid vasculitis, urticaria and angioedema

To the Editor: I read with keen interest the article published by Soldo-Juresa et al in the last issue of the journal.[1] I would like to make some comments: First, Still disease should be kept in mind whenever macrophage activation syndrome is considered in a patient with the clinical picture shown. In fact the patient's condition fulfills the criteria published by Yamagushi for Still disease.[2] I would like to remark that the measurement of soluble IL-2R (also known as CD25s) could be helpful in distinguishing between Still disease and MAS.[3] Furthermore, the development of MAS in Still disease is not infrequent and intravenous gamma globulins have shown promising results in treatment.[4,5] Second, as the authors rule out a C1q-linked vasculitis, C4 and alternative complement pathway activity should be measured. It is well known that in esterase inhibitor type II levels of C1-inhibitor esterase are normal although C4 is decreased due to low functional C1-inhibitor levels.[6] Hypocomplementemic vasculitis has been also described in patients with factor I deficiency.[7] I have attended recently a patient with vasculitis secondary to factor I deficiency. This factor was not measured by the author in this patient. Third, the authors recognize that DNA of parvovirus B-19 can persist in bone marrow for years. This is why we should distinguish between MAS secondary to viral infection or underlying Still disease that needs long-term treatment and follow-up.

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RE: Black ant stings caused by *Pachycondyla sennaarensis*: a significant health hazard

To the Editor: We read with interest the article on black ant stings by Alanazi et al[1] and would like to make the following comments. We agree with the authors that black ant stings are a significant and growing problem in Saudi Arabia. Both cases 2 and 3 had significant symptoms after black ant stings. In both cases, among other management, epinephrine was used, but it was given subcutaneously. It should be pointed out that the current recommendation is to give epinephrine intramuscularly in the lateral aspect of the thigh (vastus lateralis)[2] because there is a significant difference in the absorption of epinephrine when it is given intramuscularly in the lateral aspect of the thigh vs intramuscularly in the deltoid, or subcutaneously in the upper arm. Mean Cmax was significantly higher ($P<.01$) after epinephrine IM injection into the thigh, either from an ampule or an EpiPen, than after epinephrine IM or SC injection into the upper arm, or after saline solution IM or SC injection into the upper arm.[3]

We have a number of patients with black ant allergy and they have tested positive to an extract prepared in house. They carry two pack of Epipen and have been trained in how and when to use them. We are currently in the process of developing immunotherapy for desensitization to black ant venom. We will be very happy to accommodate any patients that need further work up/treatment for black ant allergy.

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Reply

We agree that the intramuscular route is superior to the subcutaneous in terms of better absorption and we would like to thank Dr. Gazlan for his comment.

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Fludarabine-induced bradycardia in a patient with refractory leukemia

To the Editor: A 22-year-old male diagnosed with acute myelogenous leukemia in November 2005 achieved complete remission after two courses of induction chemotherapy with idarubicin (12 mg/m^2 intravenously for 3 days) and cytosine arabinoside (200 mg/m^2 in a 24-hour infusion for 7 days). He then received an additional two courses of cytosine arabinoside (3000 mg/m^2) in a 3-hour continuous intravenous infusion twice daily for 3 days in April 2006 for intensification. Six months later, the leukemia relapsed and was refractory to re-induction chemotherapy (idarubicin 12 mg/m^2 on days 1-3 and cytosine arabinoside 200 mg/m^2 on days 1-7). With the exception of myelosuppression, no toxic events occurred during idarubicin and cytosine arabinoside treatment. Due to the refractoriness to re-induction chemotherapy, salvage chemotherapy with the FLAGI regimen (fludarabine 30 mg/m^2 on days 1-5, cytosine arabinoside 2000 mg/m^2 on days 1-5, and idarubicin 12 mg/m^2 on days 7-8) was prescribed. Before chemotherapy, his vital signs were stable, with a blood pressure of 111/65 mm Hg, a respiratory rate of 19/min, a heart rate of 97/min, and a body temperature of 36.6°C. An electrocardiogram (ECG) showed a normal sinus rhythm (Figure 1a). Premedication included dexamethasone, granisetron, and metoclopramide, which were prescribed in previous chemotherapy. Thirty minutes after the fludarabine infusion had been started, he developed sudden-onset general weakness, which lessened 5 minutes later. At that time, persistent bradycardia (48 beats per minute) was noted; vital signs were otherwise normal (blood pressure, 120/70 mm Hg; respiratory rate, 20/min; and body temperature, 36.1°C). ECG revealed sinus bradycardia but no atrioventricular block, ST segment elevation or depression, or T wave inversion (Figure 1b). The patient did not have chest tightness or pain, dizziness, cold sweats, palpitations, dyspnea, or fever with chills. Investigations showed serum potassium 4.9 mmol/L (normal, 3.5-4.5 mmol/L), creatinine 1.0 gm/dL (normal, 0.5-1.3 g/dL), CPK 19 IU/L (normal, 38-174 IU/L), creatine kinase MB fraction 2.7 U/L (normal, 3-10 U/L), and troponin I < 0.04 ng/ml (normal, <0.8 ng/mL). No specific therapy was administered; however, oxygen was given at 4 L/min via nasal cannula. The persistent bradycardia resolved gradually, 3 hours after cessation of the 5-day fludarabine infusion (Figure 1c); he then received 2 days of idarubicin treatment without incident (Figure 2).

Cytosine arabinoside is frequently used in the treatment of hematologic malignancies, especially acute myelogenous leukemia. Sporadic cases of pericarditis, pericardial effusion, cardiac tamponade, congestive heart failure, and sinus bradycardia have previously been reported as complications of cy-

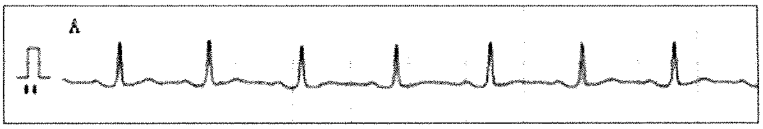

Figure 1a. ECG findings before the start of therapy.

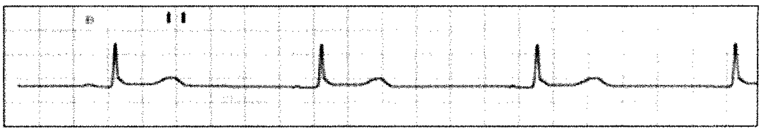

Figure 1b. ECG on day 1-5 of fludarabine infusion.

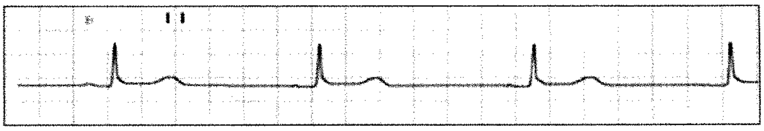

Figure 1c. ECG on the day following infusion of fludarabine.

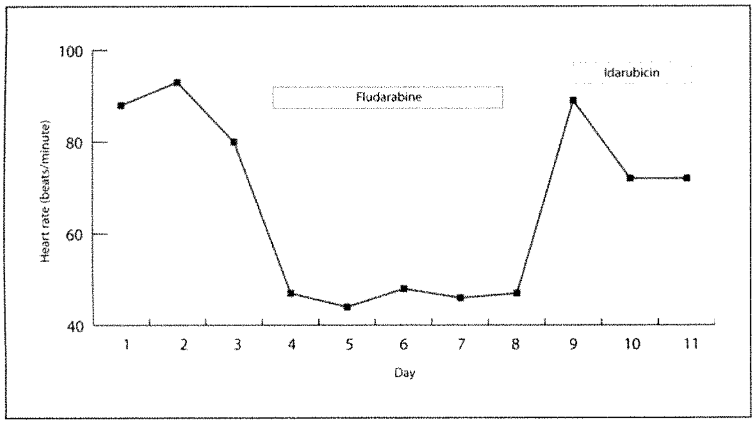

Figure 2. Correlation between heart rate and FLAGI regimen infusion.

tosine arabinoside.[1-3] Fludarabine is a purine antagonist that is commonly used for patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia. The most common adverse effects are myelosuppression and immunosuppression. Cardiovascular complications of fludarabine are rare, although several cases of congestive heart failure or left ventricular failure have been reported.[4-5]

There are many possible etiologies for the bradycardia seen in the present patient. For example, sepsis, cardiogenic events, electrolyte imbalance, and drugs such as cytosine arabinoside may all cause bradycardia. An adverse drug reaction is considered to be the probable cause of the bradycardia in this patient because no other etiologies were apparent. Cytosine arabinoside seemed an unlikely cause of bradycardia because the patient had received cytosine arabinoside-containing chemotherapy previously. The bradycardia occurred during fludarabine treatment and subsided significantly after completion of the infusion of this drug. Other probable causes of bradycardia had already been excluded and therefore it is reasonable to speculate about a possible relationship between fludarabine and the sinus bradycardia observed in this patient. Clinicians should be aware of this potential toxic effect of fludarabine, especially in view of its increased use in the treatment of hematologic diseases.

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links for authors and reviewers

Good reporting of trials and observational studies: Related guidelines and initiatives

EQUATOR

EQUATOR <**http://www.equator-network.org**> (Enhancing the QUAlity of Trials and Other Research) The EQUATOR Network is a new initiative that seeks to improve the quality of scientific publications by promoting transparent and accurate reporting of health research.

STROBE

STROBE **(STrengthening the Reporting of OBservational studies in Epidemiology)** is an international, collaborative initiative of epidemiologists, methodologists, statisticians, researchers and editors involved in the conduct and dissemination of observational studies.

CONSORT (Consolidated Standards of Reporting Trials), is part of a broader push to produce reporting guidelines (RGs) for many different types of research. The main product of CONSORT is the CONSORT Statement, which is an evidence-based, minimum set of recommendations for reporting RCTs. It offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation. The CONSORT Statement comprises a 22-item checklist and a flow diagram, along with some brief descriptive text.

ASSERT

The ASSERT **(A Standard for the Scientific and Ethical Review of Trials)** initiative is a proposed standard for the review and monitoring of randomized clinical trials by research ethics committees. Among other items, its checklist incorporates certain elements of CONSORT, to ensure fulfillment of the requirements for scientific validity in the ethical conduct of clinical research.

MOOSE

The MOOSE **(Meta-analysis of Observational Studies in Epidemiology)** paper reports the outcome of a 1997 workshop to examine the reporting of meta-analyses of observational studies. It makes recommendations to aid authors, editors, reviewers and readers of observational research.

QUOROM

The QUOROM **(Quality Of Reporting Of Meta-analyses)** conference resulted in the creation of the QUOROM Statement, which consists of a checklist and flow diagram.

STARD

The STARD **(Standards for Reporting of Diagnostic Accuracy)** initiative aims to improve the accuracy and completeness of reporting of studies of diagnostic accuracy in order to allow readers to assess the potential for bias in a study and to evaluate the generalisability of its results.

King Faisal Specialist Hospital & Research Centre

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27-28 Jumada Al Awal 143

Academic & Training Affairs
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To develop and maintain a directory of conferences and meetings organized by government institutions, academic bodies and other non-profit professional organizations in the Region.

The directory will:
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For interested applicants, please contact:
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announcements

Hepatobiliary-Pancreatic Surgery Fellowship Program

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تحديد القيم القطعية لارتفاع البطن وترابطها مع المؤشرات التقليدية للبدانة لدى الأطفال واليافعين العرب

مقدمة: البدانة وخاصة بدانة الأطفال شائعة في الشرق الأوسط ولكن لا توجد دراسات حول علاقة القطر السهمي للبطن بالواسمات التقليدية للبدانة في هذه المنطقة. فهذه الدراسة هي الأولى التي تُوثق ترابط القطر السهمي للبطن مع قياسات البدانة لدى الأطفال واليافعين العرب.

المرضى والطرق: ضمت هذه الدراسة عرضية المقطع تسعمائة وأربعة وستين فردا سعوديا أعمارهم ٥-١٧ سنة موزعين على الآتي: ٣٦٥ في سن ما قبل البلوغ (١٤٦ ولدا و٢١٩ بنتا)، و٢٤٩ بالغا (١٢٥ ولدا و١٢٤ بنتا)، و٣٥٠ في سن ما بعد البلوغ (١٩٨ ولدا و١٥٢ بنتا).

النتائج: كان هناك ترابط هام بين القطر السهمي للبطن ومؤشر البدانة بصرف النظر عن الجنس، ولكنه كان أقوى لدى الأولاد البالغين. كانت القيم القطعية كما يلي: الأفراد في سن ما قبل البلوغ ١٤ سم (يعادل الرتبة المئوية الخمسين للبنات والرتبة المئوية الستين للأولاد)؛ والأفراد في سن البلوغ ١٥ سم للبنات (الرتبة المئوية الثلاثين) و١٦ سم للأولاد (الرتبة المئوية الخمسين)؛ والأفراد بعد سن البلوغ ٢١,٥ سم للبنات (الرتبة المئوية السبعين) و٢٢ سم للأولاد (الرتبة المئوية الثمانين).

الاستنتاج: القطر السهمي للبطن مؤشر موثوق للبدانة الحشوية لدى الأطفال العرب وخصوصا اليافعين منهم. ويجب إجراء دراسات مستقبلية لتحديد ما إذا كان مثل هذا الترابط يمكن يُترجم إلى عامل خطورة واعد للإصابة بالمرض كمرض السكري ومرض القلب الإكليلي.

مدى انتشار زيادة الوزن والسمنة لدى الأطفال والمراهقين في المملكة العربية السعودية

مقدمة: هناك معلومات محدودة عن زيادة الوزن والسمنة لدى الأطفال والمراهقين في المملكة العربية السعودية. الهدف من هذه الدراسة معرفة مدى انتشار زيادة الوزن و السمنة لدى الأطفال والمراهقين في المملكة العربية السعودية.

المرضى والطرق: تم استخدام مجموعة البيانات المرجعية السعودية لعام ۲۰۰٥م لحساب مؤشر كتلة الجسم BMI لدى الأطفال من سن ٥ إلى ١٨ عاماً. وباستخدام مرجع منظمة الصحة العالمية WHO لعام ۲۰۰۷م. فقد تم تحديد نسبة انتشار زيادة الوزن والسمنة و السمنة المفرطة لدى الأطفال ذوي مؤشر كتلة الجسم بمتوسط معياري أكثر من +۱، +۲ و+۳. على التوالي. وتم استخدام مرجع عام ۲۰۰۰ من مركز مكافحة الأمراض والوقاية منها CDC للمقارنة.

النتائج: وجدنا ۱۹۳۱۷ طفلاً سليماً ومراهقاً من سن ٥ إلى ١٨ عاما. ٥٠,٨٪ منهم كانوا من الذكور. وكان معدل الانتشار الإجمالي لزيادة الوزن والسمنة و السمنة المفرطة لدى جميع المجموعات العمرية ۲۳,۱٪، ۹,۳٪ و۲٪. على التوالي. أي نسبة انتشار أقل بشكل واضح لزيادة الوزن (۲۳,۸ مقابل ۲۰.٤؛ P‹۰.۰۰۱) والسمنة (۹,٥ مقابل ٥,۷؛ P‹۰.۰۰۱) تم اكتشافها عند استخدام مرجع مركز مكافحة الأمراض والوقاية منهاCDC.

الاستنتاج: يضع هذا التقرير أسس قاعدة بيانات وطنية لمعدلات انتشار زيادة الوزن والبدانة و البدانة المفرطة لدى الأطفال والمراهقين في المملكة العربية السعودية. و يشير إلى مستويات متوسطة ما بين الدول النامية والدول الصناعية. يجب اتخاذ الإجراءات اللازمة لتجنب زيادة أعداد زائدي الوزن لدى أطفال المدارس والمراهقين والمشاكل الصحية الخطيرة المترتبة عليها

تنشيط العامل السابع المأشوب في السيطرة على النزف لدى المرضى غير النزفيين

مقدمة: هناك تقارير حديثة حول نجاح استخدام العامل السابع المأشوب (rFVIIa) مع المرضى غير النزفيين الذين خاضوا تجربة فقدان كميات كبيرة من الدم نتيجة لأذى مع تلف شديد في الأعضاء والذين تلقوا عمليات نقل دم متعددة مع تغيرات إرقائية غير ناجحة. كما أن وقت إعطاء الدواء، والجرعة، والبقاء على قيد الحياة، ووحدات نقل الدم المحفوظة، ومخاطر حدوث تخثر إضافة إلى نسب المخاطر/ الفوائد مازالت لم تعرف بشكل جيد.

المرضى والطرق: أجريت دراسة مراجعة حول جميع التقارير الطبية للمرضى الذين عولجوا باستخدام العامل السابع المأشوب (rFVIIa) بين يناير ٢٠٠٣م ومارس ٢٠٠٨م. المعلومات التي تم تجميعها تضمنت السمات الديموغرافية، التشخيص، دواعي الإستعمال، الأمراض المصاحبة، منتجات الدم المستخدمة مع العامل السابع المأشوب (rFVIIa)، جرعة العامل السابع المأشوب (rFVIIa)، البقاء على قيد الحياة، والمضاعفات.

النتائج: قمنا بتحديد ٤٥ مريض، ٢٧ (٦٠٪) ذكور و ١٨ (٤٠٪) إناث، بمتوسط عمر ٥٢ سنة. ومتوسط جرعة العامل السابع المأشوب (rFVIIa) ٤٠ ميكروغرام/كيلوغرام (تتراوح ما بين ٢٠ إلى ١٢٠ ميكروغرام/كيلوغرام). خمسة مرضى (١١,١٪) احتاجوا إلى جرعة ثانية من العامل السابع المأشوب (جرعة تتراوح ما بين ٢٠ إلى ٨٥ ميكروغرام/ كيلوغرام) بينما احتاج ثلاثة مرضى (٦,٧٪) جرعة ثالثة من العامل السابع المأشوب (rFVIIa) (جرعة تتراوح ما بين ٤٠ إلى ٦٠ ميكروغرام/ كيلوغرام). كان هناك انخفاض ملحوظ وواضح في نقل عبوات خلايا الدم الحمراء (P= .٠٠٧٨). وبشكل عام، كان هناك انخفاض في نقل الدم بعد حقن العامل السابع المأشوب (rFVIIa) (p)= .٠٣٢٣). وتوفي تسعة عشر مريضاً (٢٤,٢٪) وأصيب ٣ مرضى بتخثر (٦,٧٪).

الاستنتاج: ينبغي أن يعتمد استخدام العامل السابع المأشوب (rFVIIa) على دلائل سريرية راسخة لإيجاد توازن ما بين المخاطر والفوائد والتكلفة إذا ما تم استخدامه للمرضى غير النزفيين. نحتاج إلى دراسات مستقبلية عشوائية للتحقق من الفعالية مقابل التكلفة للعامل المأشوب (rFVIIa) لدواعي الإستعمال والتوصل إلى تقييم نهائي حول أهمية هذا العلاج.

داء الرشاشيات الرئوي الغازي في حالات التفاقم الحاد للمرض الرئوي الانسدادي المزمن والقيمة التشخيصية لدمج الفحوص المصلية

مقدمة: هناك زيادة في تكرار الإصابة بداء الرشاشيات الرئوي الغازي لدى من يعانون من المرض الرئوي الانسدادي المزمن. أجرينا هذه الدراسة لاكتشاف معدل الإصابة التقريبي لإصابات داء الرشاشيات الرئوي الغازي في حالات التفاقم الحاد للمرض الرئوي الانسدادي المزمن. ولمعرفة ما إذا كان دمج نوعين من الفحوص (فحص مستضد غالاكتومانان galactomannan [MG] antigen وفحص اكتشاف ١. ٣ • بيتا- جلوكان ١, ٣ - B - glucan [BG] detection) سيؤدي إلى تشخيص أدق لداء الرشاشيات الرئوي الغازي.

المرضى والطرق: تضمنت الدراسة ٢٦١ مريضاً عانوا من تفاقم حاد للمرض الرئوي الانسدادي المزمن وتم تنويمهم في المستشفى على مدار عامين. وتم تصنيف المرضى وفقاً للتعريفات المعدّلة لداء الرشاشيات الرئوي الغازي. تم الكشف عن مستضد غالاكتومانان MG antigen و بيتا جلوكان BG من خلال فحوص بلاتيليا أسبيرغيليس Platelia *Aspergillus* و غلوكاتيل Glucatell.

النتائج: ثبت أن هناك مريضين يعانيان من داء الرشاشيات الرئوي الغازي، وثلاثة إصابتهم مرجحة واثنين إصابتهم ممكنة. معدل الإصابات المثبتة أو المرجحة بداء الرشاشيات الرئوي الغازي لدى من يعانون من المرض الرئوي الانسدادي المزمن كانت ١,٩١٪ (٥/٢٦١). تمت معالجة أربعة مرضى من ذوي الإصابات المثبتة والمرجحة بداء الرشاشيات الرئوي الغازي بواسطة ستيرويدات شاملة أو مستنشقة قبل تنويمهم في المستشفى. وكانت الأعراض النمطية والدلائل التشخيصية لداء الرشاشيات الرئوي الغازي أقل شيوعاً لديهم بشكل نسبي. وكان معدل الوفيات للمرضى من ذوي الإصابات المثبتة والمرجحة بداء الرشاشيات الرئوي الغازي ٨٠٪. وكان معدل دقة التشخيص لفحصي غالاكتومانان MG antigen و بيتا جلوكان BG معاً ٩٨,٨٪.

الاستنتاج: إن الإصابة بداء الرشاشيات الرئوي الغازي في حالات التفاقم الحاد للمرض الرئوي الانسدادي المزمن مشكلة متزايدة ومقلقة. ودمج نوعين من الفحوص المصلية زاد من دقة التشخيص.

تحليل الطفرات الجينات التي تتعلق بمقاومة الكلوروكين في طفيليات الملاريا المنجلية المعزولة من المرضى في جنوب غرب السعودية

مقدمة: يستخدم عقار الكلوروكين لعلاج الملاريا منذ عدة عقود. وهدفنا هو فحص الأساس الجزيئي لمقاومة الكلوروكين بين معزولات المتصورة المنجلية من المنطقة الجنوبية الغربية في السعودية من خلال تحليل كل من طفرات(kV٦T وN٨٦Y) في الجينات PFCRTو PFMDR١.على التوالي.

المرضى والطرق: تم جمع عينات الدم المصابة بالمتصورات المنجلية (n=١٢١) على أوراق ترشيحية. كما تم استخلاص الحمض النووي (DNA) بالإضافة إلى إجراء تضخيم لكسر الجينات المذكور أعلاه باستخدام اختبار التفاعل السلسلي للبوليميراز. تم هضم وتسلسل التضخمات عن طريق إنزيم Apo enzyme

النتائج: من ضمن ١٢١ عينة، تمكنا من تضخيم ٩٥ و١١٢ عينة للطفرات (PFCRT, KV٦T و PFMDR١ (N٨٦Y). على التوالي. أما بالنسبة لكل العينات التي تم تضخيمها لطفرة PFCRT KV٦T، فإنه لم يتم هضمها عبرApo enzyme مما يوحي على وجود طفرة KV٦T. أما بخصوص طفرة PFMDR١ N٨٦Y، فقد تم هضم ١٠٩/٦٥ (٥٩,٦٪) من العينات وذلك عندما تم علاجها بواسطة (Apol) على نحو نمطي، مما يوحي على وجود مادة الأليل الجامح (N٨٦) (Wild allele). وعلى الرغم من ذلك، فقد تم هضم ١٠٩/٤٤ (٤٠,٤٪) من العينات بواسطة (Apol)، مما يوحي على وجود طفرة أليل (Y) في الموقع ٨٦. وقد أكد فحص تسلسل الحامض النووي هذه النتائج.

الاستنتاج: تفاجئنا بأن كل المعزولات أظهرت وجود الطفرة أليل في الرامزة ٧٦ (TV٦N) للطفرة PFCRT. ولكن، تم العثور على الطفرة أليل المتحورة في الرامزة ٨٦ (N٨٦Y) للطفرة PFMDR١ في ٤٠,٤٪ من العينات المدروسة. ولعلمنا، فهذه هي الدراسة الأولى استقصت وجود الطفرة في الجين PFMDR١ في المملكة. وستساهم هذه الدراسة على تطوير استراتيجيات التدخل العلاجي ضد الملاريا في السعودية.

الخلاصات العربية

المدونات الطبية السعودية

تصدر المدونات الطبية السعودية مرة كل شهرين عن مستشفى الملك فيصل التخصصي ومركز الأبحاث . ص.ب ٣٣٥٤ . الرياض ١١٢١١. المملكة العربية السعودية. يتم توزيع المدونات وإعداد الملخصات العربية بالتعاون مع د. زهير القاوي بواسطة موظفي إدارة الخدمات الإدارية بمستشفى الملك فيصل التخصّصي ومركز الأبحاث. جميع المراسلات والاستفسارات وطلبات الاشتراك ترسل إلى المحرر الأول مكتب المطبوعات (ر.إ ٣٦).

سعر الاشتراك ١٠٠ ريال في السنة للأفراد داخل المملكة و٢٠٠ ريال في السنة للأفراد خارج المملكة و٥٠٠ ريال في السنة للمؤسسات. يمكن فتح اشتراك مجاني للأفراد و المؤسسات في الدول النامية.

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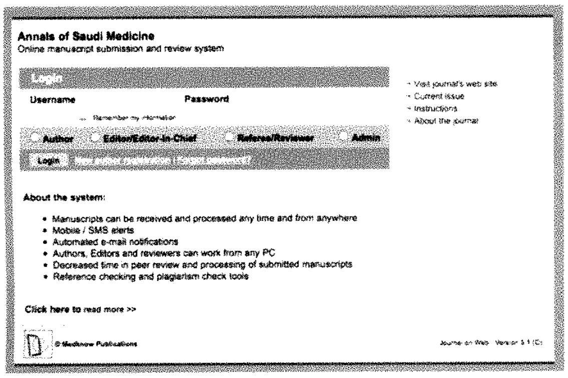

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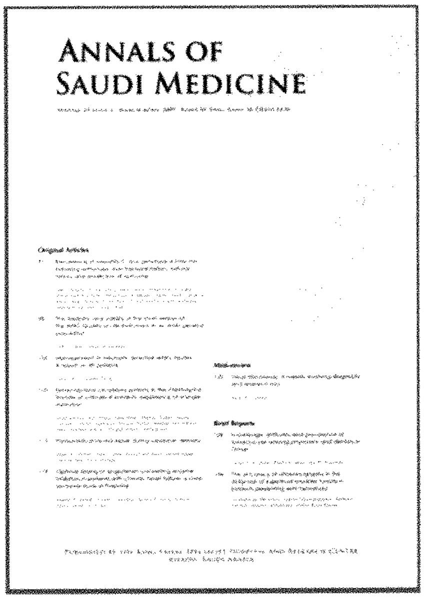

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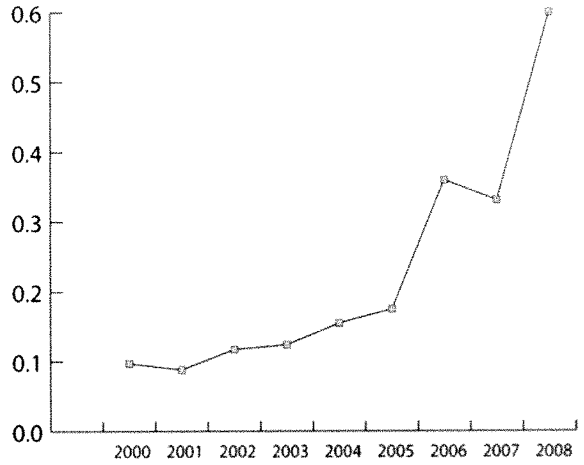

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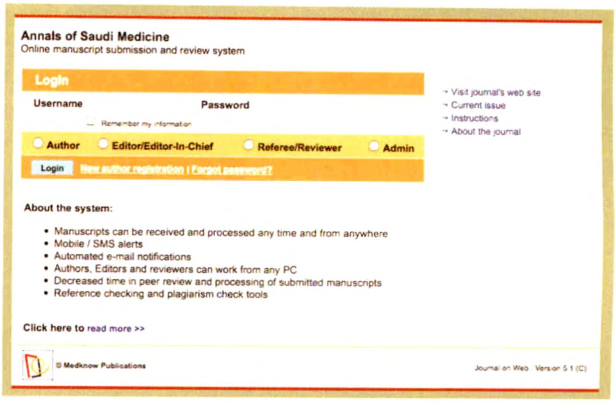

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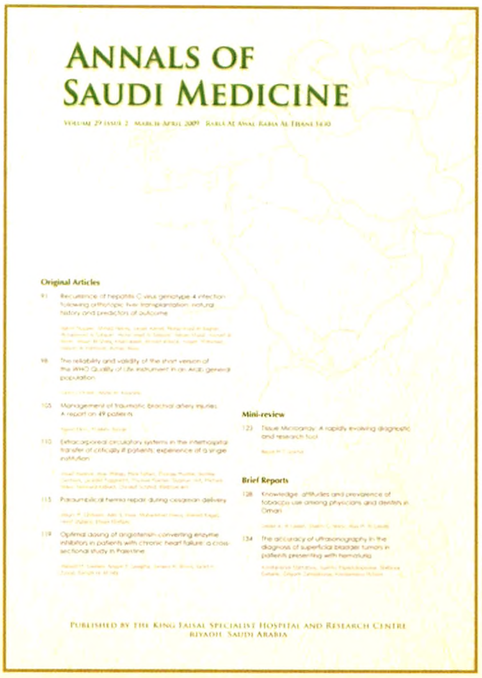

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1432-33/2011

ACADEMIC CALENDAR

JANUARY

The Advancement of Blood Management Symposium
12-13 January 2011 / 08-09 Safar 1432
Contact No.: 464-7272 Ext. 32680/32700 (Anesthesiology)

4th KFSH&RC Residents' Research Day
19 January 2011 / 15 Safar 1432
Contact No.: 464-7272 Ext 31730 (Research Center)

2nd Saudi-Belgian Robotic Surgery Symposium
TBA
Contact No. 442-4302 (Urology)

FEBRUARY

Coblation Course
08-09 February 2011 / 05-06 Rabi Al Awal 1432
Contact No. 464-7272 Ext. 23425 / 27799 / 32474 (ORL)

Urodynamic Course
12 February 2011 / 09 Rabi Al Awal 1432
Contact No. 442-4302 (Urology)

1st Pediatric Flexible Bronchoscopy Course
21-23 February 2011 / 18-20 Rabi Al Awal 1432
Contact No. 464-7272 Ext. 31960 (Pediatrics)

MARCH

Intensive Microsurgery Hands-On Course
05-09 March 2011 / 30 Rabi I - 04 Rabi II 1432
Contact No. 442-7773 (Neurosciences)

Combating Antibiotic Resistance Course
08-09 March 2011 / 03-04 Rabi Al Thani 1432
Contact No. 464-7272 Ext. 38628 (AUE)

International Skeletal Society Regional Outreach Program
15-17 March 2011 / 10-12 Rabi Al Thani 1432
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3rd Cleft Lip & Palate Symposium
22-24 March 2011 / 17-19 Rabi Al Thani 1432
Contact No. 442-7652 (Dentistry)

Annual Saudi Colorectal Surgery Forum
26-30 March 2011 / 21-25 Rabi Al Thani 1432
Contact No.: 442-7754 (Surgery)

APRIL – MAY

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09-10 April 2011 / 05-06 Jumada Al Awal 1432
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Sleep and your Health Symposium & Workshop
13-14 April 2011 / 09-10 Jumada Al Awal 1432
Contact No.: 442-7494 (Medicine)

The 2nd Annual Medical Trainee Research Day
20 April 2011 / 16 Jumada Al Awal 1432
Contact: 442-7495 (Medicine)

3rd Saudi Allergy, Asthma & Clinical Immunology Symposium
26-27 April 2011 / 22-23 Jumada Al Awal 1432
Contact No.: 464-7272 Ext. 31830 or 442-7763 (Pediatrics)

Intensive Microsurgery Hands-On Course
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Contact No. 442-7773 (Neurosciences)

Oncology Centre Academic & Research Day
04 May 2011 / 01 Jumada Al Thani 1432
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13th Annual Research Celebration Day
10 May 2011 / 07 Jumada Al Thani 1432
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Towards Safer Neurosurgery Symposium
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9th Annual Academic Day
25 May 2011 / 22 Jumada Al Thani 1432
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4th Pediatric Infectious Diseases Seminar
31 May - 01 June 2011 / 28-29 Jumada Al Thani 1432
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Research by Medical Trainees: Current Status & Future Planning Workshop
14-15 June 2011 / 12-13 Rajab 1432
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NOVEMBER & DECEMBER

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Contact No. 442-7773 (Neurosciences)

Histocompatibility and Immunogenetics Workshop
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Maternal Medicine Symposium
22-23 November 2011 / 26-27Dhu Al Hijja1432
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Advanced Laparoscopic Hands-On Course
30 Nov - 01 Dec 2011 / 05-06 Muharram 1433
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8th Annual AIDS Workshop
03 December 2011 / 08 Muharram 1433
Contact No. 442- 8494 (Medicine)

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05-08 December 2011 / 10-13 Muharram 1433
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Future of Prevention of Genetic Diseases in KSA: New KFSH&RC Initiative Workshop
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Contact No.: 442-4988 (Medical Genetics)

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Ministry of Defense and Aviation
Armed Forces Medical Services General Directorate

Under the Aegis of HRH Prince Khalid Bin Sultan Bin Abdulaziz Al-Saud

The Assistant To The Minister of Defense and Aviation and Inspector General for Military Affairs

The Second International Congress of PAN-ARAB Regional Group of Military Medicine

Held in the KINGDOM OF SAUDI ARABIA - Jeddah (4-9 December 2010)

1. **Main Topics**

A. Armed Forces Health Protection:
- NBC.
- Preventive medicine, industrial medicine, and hygiene in a military environment.
- Techniques for assessing physical and mental aptitudes required for various military functions.
- Employment health.
- Military occupational health.
- Infectious diseases of military importance.
- Military ergonomics.
- Hygiene of water and food.
- Zoonosis prevention or relation to animal physiopathology in a military environment.
- Activities of veterinary sciences when such activities contribute to the forms of humanitarian missions.
- Diving medicine (diving accident).
- Aviation medicine.
- Dental and oral medicine in a military environment.
- Pharmacy in a military environment.

B. Medical support in combat environment:
- Chemical, biological, nuclear, radiation and explosives.
- Navy.
- Aviation.
- Desert.
- Cold climate.
- Altitude.
- Rural areas.
- Counter insurgency warfare.
- Airborne operations.
- Amphibious operations.
- Medical logistic at operational field.
- Pharmacy.
- Activities of veterinary sciences when such activities contribute to the forms of battle missions.
- Medical evacuation.
- Combat medicine and traumatology.
- Medical rehabilitation.

C. Humanitarian missions:
- Cooperation between Armed Forces Medical Services Directorates and other national or international medical organizations/institutes.
- Rapid response medical teams.
- Natural mass disaster.
- Medical support in case of massive crowds (Religious, cultural, political or sporting events).

D. Field administrative activities:
- Role of medical intelligence (pre-deployment).
- Role of telecommunication in military medicine.
- Role of total quality management in Military operational field.
- Field operational medical planning.
- Medical ethics in armed conflicts.
- Surveillance in all military medical matters.

2. **Workshops:**
- Evidence based medicine
- Research methodology
- Veterinary science

3. **Round Tables:**

Dentistry:
Subject: 1. Frequency of Oral Pathologic Findings in Recruits.
Subject: 2. DNA Application to the Forensic Arena/21st Century Technologies.

Veterinary Sciences:
Subject: Early detection of emerging diseases.

Pharmacy:
Subject: Medical supply of deployments.

Education and Training:
Subject: Center of reference for education on International Humanitarian Law and Medical Ethics.

Administration and Logistics:
Subject: Medical Planning of Military Operations.

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K469E polymorphism of the intercellular adhesion molecule-1 gene in Egyptians with coronary heart disease

Mohamed and colleagues found a higher frequency of the K genotype of the *ICAM-1* gene in 100 patients with CHD and a 3.8 times increased risk of disease in this case-control study. Serum ICAM-1 levels were similar in cases and controls, and are not necessarily elevated in CHD.

OSTEOPOROSIS

Association of interleukin-1 beta (-511C/T) polymorphisms with osteoporosis in postmenopausal women

Chao and colleagues found statistically significant differences in the frequencies of IL-1β but not IL-1α and IL-1 receptor antagonist genes in 117 postmenopausal women with osteoporosis compared with normal control subjects without a history of symptomatic osteoporosis. The authors conclude that the IL-1β (-511C/T) is an independent risk factor for osteoporosis.

IN VITRO FERTILIZATION

Comparing serum basal and follicular fluid levels of anti-Müllerian hormone as a predictor of in vitro fertilization outcomes in patients with and without polycystic ovary syndrome

In a prospective study, Arabzadeh and colleagues found that serum basal AMH and FF levels were significantly higher in women with polycystic ovary syndrome vs controls. In the control group, there was a positive relationship between serum basal AMH levels and the percentage of matured oocytes and implantation rate, indicating that AMH levels may be predictive of IVF outcomes in normo-ovulatory but not polycystic ovary syndrome patients.

DERMATOLOGY

The pattern of skin diseases in the Qassim region of Saudi Arabia: What the primary care physician should know

In a prospective study over a 12-month period, Shobalili found that the most common skin diseases in 3051 patients in the Qassim region were eczema/dermatitis, viral infections, pilosebaceous disorders, pigmentary lesions and hair disorders. He concludes that training programs are needed for primary care physicians to reduce referrals to dermatology clinics.

DIABETES

Glycemic changes after vitamin D supplementation in patients with type 1 diabetes mellitus and vitamin D deficiency

Aljabri and colleagues found that vitamin D supplementation improved glycemic control in vitamin D replete, type 1 diabetes patients. There were significant differences in mean glycosylated hemoglobin levels after 12 weeks of supplementation between groups that achieved different tertiles of 25-hydroxyvitamin D levels.

SURGERY

Effect of classic uvulopalatopharyngoplasty and laser-assisted uvulopalatopharyngoplasty on voice acoustics and speech nasalance

In 27 patients with obstructive sleep apnea who underwent UPPP for oropharyngeal reconstruction by either the classic or laser-assisted technique, there were no differences in auditory perceptual assessment of voice and speech or objective assessment using acoustic voice analysis and nasometry.

perspective

Permissibility of prenatal diagnosis and abortion for fetuses with severe genetic disorder: type 1 spinal muscular atrophy

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Abortion has been largely avoided in Muslim communities. However, Islamic jurists have established rigorous parameters enabling abortion of fetuses with severe congenital abnormalities. This decision-making process has been hindered by an inability to predict the severity of such prenatally-diagnosed conditions, especially in genetic disorders with clinical heterogeneity, such as spinal muscular atrophy (SMA). Heterogeneous phenotypes of SMA range from extremely severe type 1 to very mild type 4. Advances in molecular genetics have made it possible to perform prenatal diagnosis and to predict the types of SMA with its potential subsequent severity. Such techniques will make it possible for clinicians working in predominantly Muslim countries to counsel their patients accurately and in harmony with their religious beliefs. In this paper, we discuss and postulate that with our current knowledge of determining SMA types and severity with great accuracy, abortion is legally applicable for type 1 SMA.

Historically the Muslim community has not permitted abortion except under exceptional circumstances strictly regulated by the consensual fatwa of religious scholars.[1] These circumstances include cases of severe congenital abnormalities incompatible with life. Rigorous preconditions are put in place to avoid potential abuse. The preconditions are those related to the state of the disease and the diagnostic procedures involved. Factors related to the disease include certainty of death within a short period of life and poor quality of life for the family. Diagnostic and confirmative procedures include the need to involve a panel of at least three experts in establishing the diagnosis, a strict time line of 120 days (or 134 days from the last menstrual period) from conception when the termination should be done, and the requirement that the request for prenatal diagnosis and termination must come from both parents.

Islamic teachings consider the day 120 from conception as an important milestone prior to a period of 'ensoulment' of a developing fetus. Fetuses severely affected by conditions such as trisomy 13, 18, anencephaly, bilateral renal agenesis, Tay-Sachs disease are allowed to be terminated within this period. Abortion for serious congenital anomalies is legal in some Islamic countries such as Kuwait, Tunisia, Turkey and Jordan.[2,3] However, the rulings and permissibility are complicated by the lack of a reliable predictor of seriousness and the fatality of the condition in question. This situation is made worse when faced with a genetic abnormality with a wide spectrum of clinical heterogeneity, such as spinal muscular atrophy (SMA). This paper attempts to simplify this dilemma by offering a solution to issues of predicting severity of SMA through the use of molecular genetics analysis.

Spinal muscular atrophy

SMA is a common autosomal recessive neuromuscular disorder characterized by weakness of the proximal limb and trunk muscles. According to the disease severity and age of onset, SMA is classified into four clinical types.[4] In 1995, Lefebvre et al[5] demonstrated that 93% of SMA cases carry a single kind of mutation—deletion of *SMN1*, a gene located in 5q13, where all these four clinical types were mapped. Molecular analysis of SMA (i.e., identification of *SMN1* deletion) has been a method of choice in the diagnosis of the disorder[6] and for prenatal genetic diagnosis.[7] Molecular diagnosis of SMA has become relatively easy, inexpensive and is comfortable and accessible worldwide. Molecular analysis, including prenatal genetic diagnosis, of SMA has recently been reported in countries with a predominantly Muslim population, such as Saudi Arabia,[8] Tunisia,[9] Iran,[10] Malaysia[11] and Turkey,[12] which indicates that Muslim countries have become largely exposed to advanced methodologies that enable easy and fast molecular diagnosis of SMA.

SMA type 1 and fulfillment of criteria for termination of pregnancy in Muslim communities

The *fatwa*[1] (or edict), as briefly outlined above, imposes two conditions for the eligibility for termination of pregnancy for congenital abnormalities: (1) that the condition is certain to cause death within a short period of life and (2) that the condition would result in poor quality of life for members of the family. SMA type 1 represents the most severe type of SMA. Its onset is usually during the prenatal period, immediately after birth or within 6 months of life. Weakness of the diaphragm combined with weakened intercostal muscles is the predominant cause of respiratory failure. Tongue fasciculation and weakness due to bulbar denervation cause poor sucking and swallowing, as well as decrease airway protection, increasing the risk of aspiration pneumonia, which is an important cause of mortality. Eighty percent of babies affected by SMA type 1 die by 1 year of age, 100% by 2 years, and some with extreme severity die within days of birth.[13-15] The quality of life of affected patients is poor.[16] An inability to communicate their feelings of discomfort, pain or suffering, especially in response to uncomfortable or painful medical intervention, and inability to cough, clear secretions or swallow contribute to this poor predicament.[17] **Table 1** shows how SMA type 1 prevails in fulfilling the criteria for abortion, compared to other SMA types. These descriptions constitute conditions in which death certainly occurs within a short a period of life in all patients with SMA type 1 and where the family's life will be significantly affected.

Determination of SMA severity by molecular genetic diagnosis

Fetuses with *SMN1* deletion may not manifest the expected clinical severity postnatally. As such, the challenge is to identify genetic markers that would accurately predict the clinical course after the fetus is born. This dilemma was solved by studies that showed that the SMA phenotype is directly linked to the size of the genomic deletion.[18] Researchers have discovered disease-modifier genes—the *SMN2* and *NAIP*—that are located adjacent to *SMN1* gene within a duplicative 5q region.[19,20] These observations have been demonstrated by studies conducted among Japanese, Vietnamese, Malaysian and Western ethnic populations.[19-24] **Table 2** shows that when *SMN1* deletion occurs concurrently with a single copy of *SMN2*, the patients always manifest the severe type of SMA, regardless of *NAIP* status. However, when two copies of *SMN2* are present in *SMN1*-deleted patients, the clinical severity is slightly variable, in which case, *NAIP* deletion status can help determine the clinical severity.

Table 1. Comparison on the extent of disability, treatment and support group among SMA types[4,26,27]

	SMA type I	SMA type II/III/IV
Life expectance	≤ 2 years (none survived beyond 2 years of age)	Adolescence/adulthood /adulthood
Prenatal diagnosis before 120th day	Available	Available
Prenatal determination of severity before 120th day	Available	Not available
Risk to life-threaning diseases soon after birth	Respiratory insufficiency (100%)	Respiratory insufficiency (<15%)
Life productivity	None	Productive; limited physical activity
Development of self-esteem	None	Present
Availability of social support group	Limited to developed countries	Limited to develop cuntries
Availability of treatment	None prolonging life	Supportive
Independence	None	Present with limitation

The diagnostic pathway for the fulfillment of criteria for termination of pregnancy in a Muslim community

Diagnostic and confirmative procedures require fulfillment of three conditions for termination of pregnancy due to congenital abnormalities: (1) the diagnosis should be confirmed by at least three experts with expertise in the related field; (2) the prenatal diagnosis and termination should be done before day 120 of conception or before the 134th day from the last menstrual period (~17 weeks); and (3) the request for prenatal diagnosis and termination must come from both parents. Specimen sampling for prenatal genetic diagnosis can be performed at 9-11 weeks of gestation from chorionic villi[25]; or at 10-12 weeks, from amniotic fluid[10]; so that the time period is sufficient to allow a complete diagnostic procedure.

We propose a diagnostic pathway to assist clinicians in deciding on the appropriateness of termination of pregnancy once a fetus is found to carry the *SMN1* deletion. This decision-making process takes into account the copy number variation of the disease-modifying genes, which will make prediction of severity accurate, allowing termination of pregnancy within the time period permissible by jurists in the Muslim community (**Figure 1**). **Figure 1** outlines the flow of the diagnostic pathway we propose for determining the severity of SMA. When both parents request a prenatal genetic diagnosis, it is important to make sure that the index case is clinically SMA type 1 with deletion of the *SMN1* gene. It is also crucial to initially determine that the gestational age is younger than 120 days and that the time period is enough to allow complete diagnostic procedure.

Following fetal specimen sampling and subsequent DNA extraction, the *SMN1* deletion test is performed on the fetal DNA. Microsatellite marker analysis is performed to rule out the possibility of contamination with a maternal specimen. This is especially important in the case when no *SMN1* deletion is proven. In this case, clinical reassessment is suggested if there is no indication of maternal contamination. Re-sampling of fetal specimen is required if there is an indication of maternal contamination. If *SMN1* deletion is proven, the test would go further, into *SMN2* copy number analysis. A single copy of *SMN2* directly determines that the fetus will have a type 1 clinical severity of SMA. More than two copies of *SMN2* disable severity determination. Two copies of *SMN2* require *NAIP* deletion analysis, whereby only if the gene is deleted, can SMA type 1 be determined. Therefore, SMA type 1 can be determined based on only two genotypes (*SMN1-SMN2-NAIP*):

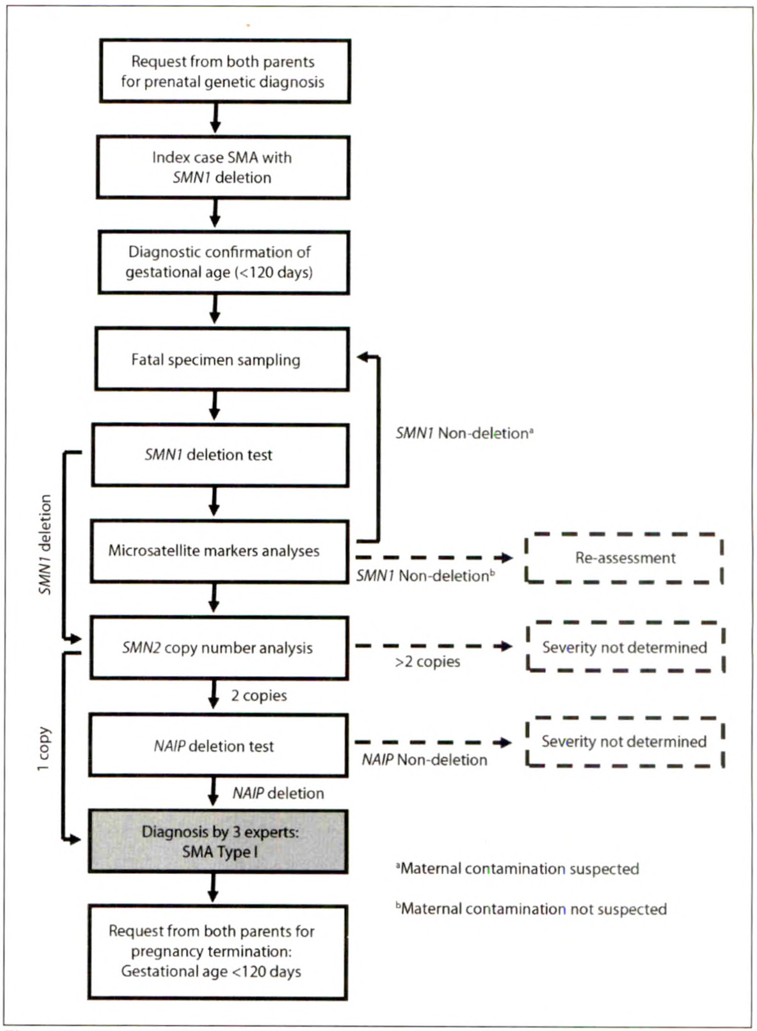
Request from both parents for prenatal genetic diagnosis
Index case SMA with SMN1 deletion
Diagnostic confirmation of gestational age (<120 days)
Fatal specimen sampling
SMN1 deletion test
Microsatellite markers analyses
SMN2 copy number analysis
NAIP deletion test
Diagnosis by 3 experts: SMA Type I
Request from both parents for pregnancy termination: Gestational age <120 days
SMN1 Non-deletion[a]
SMN1 Non-deletion[b]
Re-assessment
>2 copies
Severity not determined
2 copies
NAIP Non-deletion
Severity not determined
NAIP deletion
SMN1 deletion
1 copy
[a]Maternal contamination suspected
[b]Maternal contamination not suspected

Figure 1. Flowchart describing the pathway of prenatal diagnostic procedures in determining SMA diagnosis and severity.

Table 2. Prediction of SMA severity using enotypic pattern of SMA locus.

Genotype	Severe	Milder	Total
SMN1-SMN2-NAIP[a]	Type I	Types II and III	
0-1-≥0[19,20,22-24]	25	0	25
0-2-0[20,21,23]	14	0	14
0-2-unknown[19,24]	181	15	196
0-2->0[20,21,23]	14	12	26
0->2-0[20,21,23]	0	5	5
0->2->0[20,21,23]	5	48	53
Total	**239**	**80**	**319**

[a]SMN1: 0=homozygous deletion; SMN2: 1=1 copy; 2=2 copies; > 2=more than 2 copies; NAIP: 0=homozygous deletion; > 0=no homozygous deletion.

0-1 or 0-2-0. The diagnosis of SMA type 1 should be decided by consensus among a panel of at least three experts. Finally, termination of pregnancy should be based on a request from both parents after the final decision is made, provided the gestational age is still under 120 days.

The above diagnostic pathway should, however, be interpreted with caution in fetuses with deletion of only *SMN1* exon 8, when the *SMN1* exon 7 is found intact. This caution is of importance since only a genetic alteration within exon 7 has been proven to cause SMA.[28] Previous studies have shown variable severity among individuals with more than one *SMN2* copies and non-deletion of *NAIP*. It is therefore not recommended to determine the severity if the fetus has a genotypic pattern other than those described within **Figure 1**.

Conclusions and Recommendations

To the best of our knowledge, this is the first scientific approach that addresses the possibility of prenatal genetic diagnosis and abortion for congenital anomalies with high clinical heterogeneity for Muslim communities. This paper addresses an important dilemma in the Muslim community—in as much as early termination of pregnancy in case of a fatal disorder is allowed in the Islamic religion; however, previous scientific knowledge had not addressed accurately the genetic markers which are present on an unborn fetus that will accurately predict the fatality of the disease.

We have outlined the most recent findings, which indicate that the most severe type of the disease that most commonly results in fatality can in fact be diagnosed prenatally through the genomic deletion affecting *SMN1* gene and the copy number variations of disease-modifier genes, the *SMN2* and *NAIP*. Termination of such fetuses can then be permissible and done according to the laws of the Muslim community. The clinicians responsible for decision making and the parents who wish to terminate the pregnancy are therefore in a safe position, from the religious point of view. This knowledge, when made available in such communities, should lead to greater acceptability and will stand to benefit a vast number of people of the Muslim faith. It will also serve as a useful guide to clinicians working in the Muslim community and facing this dilemma.

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original article

K469E polymorphism of the intercellular adhesion molecule-1 gene in Egyptians with coronary heart disease

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BACKGROUND AND OBJECTIVES: The initial step in atherosclerosis is the adhesion of leukocytes to activated endothelial cells mediated by intercellular adhesion molecule-1 (ICAM-1). This study aimed to investigate the association of K469E polymorphism of the *ICAM-1* gene and soluble ICAM-1 (sICAM-1) serum level with coronary heart disease (CHD) in Egyptian subjects.

PATIENTS AND METHODS: Using a case-control design, we studied 100 patients with CHD, including 73 patients with acute myocardial infarction (MI) and 27 with unstable angina (UA). The control group consisted of 50 healthy subjects with normal left ventricular function. All participants were genotyped for the ICAM-1 polymorphism by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Serum sICAM-1 was measured by enzyme-linked immunoassay (ELISA).

RESULTS: In CHD patients, the frequencies of K genotype (KK and EK) were significantly higher when compared to controls (P<.001) and were associated with an increased risk of disease development (OR=3.8, 95% CI: 1.7 to 8.5; P=.001). K genotype frequencies in patients with MI showed no significant difference when compared to patients with UA (P= .121). Serum sICAM-1 levels were comparable between CHD patients and controls (P= .37) and between MI and UA patients (P=.23). There were no significant differences in sICAM-1 levels among patients with different genotypes (P=.532). Men presented with higher sICAM-1 levels than women (P=.004).

CONCLUSION: ICAM-1 gene polymorphism in codon 469 is associated with a risk for CHD development in Egyptian subjects. Serum sICAM-1 is not influenced by this polymorphism and is not necessarily elevated in CHD.

Cardiovascular disease (CVD) remains the leading cause of death in the developed world. Although hyperlipidemia screening and treatment has proven to be one of the most effective tools for reducing CVD burden, it often fails to identify a substantial proportion of persons at high risk for CVD-associated events. Elevations in markers of inflammation and thrombosis, such as high sensitivity C-reactive protein, soluble intercellular adhesion molecule, homocysteine and fibrinogen, are also associated with increased CVD risk.[1] Intercellular adhesion molecule-1 (ICAM-1) is an important inflammatory protein; it is one of the adhesion molecules that plays a key role in the recruitment of leukocytes at sites of inflammation and the tight adhesion between leukocytes and vascular endothelium. Inflammation plays a critical role in atherogenesis. The initial step in atherosclerosis is the adhesion of leukocytes to activated endothelial cells mediated by intracellular adhesion molecule-1 (ICAM-1).[2] Soluble ICAM-1 (sICAM-1) is a circulating form of ICAM-1 and has been shown to reflect cell surface expression of ICAM-1 in human vascular endothelial cells. The main mechanism responsible for release of sICAM-1 is believed to be proteolytic cleavage of ICAM-1 from cell membranes.[3] The *ICAM-1* gene is located on chromosome 19 p13.2-p13.3. A single-base C to T transition polymorphism, which results in an amino acid substitution from glutamine (E) to lysine (K) in the ICAM protein in exon 6 codon 469, has been found to be related to inflammatory diseases and atherosclerosis.[4] The aim of this study was to investigate the association of K469E polymorphism of the *ICAM-1* gene and sICAM-1 serum level with coronary heart disease (CHD) in Egyptian subjects.

PATIENTS AND METHODS

The study population consisted of 100 patients with acute CHD, including 73 patients with acute myocardial infarction (MI) and 27 patients with unstable angina (UA) (mean [standard deviation] age: 52.6 [8.29] years; and male/female ratio: 87/13). They were recruited from the Department of Internal Medicine and critical care unit of Cairo University Hospitals in the period from October 2008 to June 2009. The diagnosis of CHD was based on medical history, physical examination, typical electrocardiographic changes, increases in serum cardiac enzymes activities and coronary angiography. Fifty healthy subjects with normal left ventricular function constituted the control group (mean [SD] age: 53.9 [6.2] years, and male/female ratio: 38/12). Subjects with any inflammatory condition were excluded. Blood samples were collected after a fast of 12 to 14 hours from all subjects after obtaining oral consent. Serum and EDTA samples were stored at −20°C until assay time.

Assay of serum total cholesterol, HDL and triglycerides was performed on automated analyzer, Hitachi 917; commercial kits were supplied by Roche Diagnostics (Boehringer Mannheim, GmbH D-68298, Mannheim, Germany). The LDL level was calculated using the Friedwald equation. Soluble ICAM-1 levels in serum were measured by solid-phase sandwich enzyme-linked immunoassay (ELISA) kit supplied by Thermo Scientific Life Science, USA.[5]

Molecular analysis of K469E polymorphism of ICAM-1 *gene*

DNA extraction was performed using the standard salting-out technique.[6] Genotype analysis was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The sense primer was 5'-GGA ACC CAT TGC CCG AGC -3', and the antisense primer was 5'- GGT GAG GAT TGC ATT AGG TC -3'. PCR amplification of a 223-bp fragment of exon 6 was performed in 50-μL reaction mixture consisting of 67 mM Tris-HCl (pH, 8.8), 1 mM magnesium chloride, 10 mM dNTPs, 5 pmol of each primer, 100 ng of extracted DNA and 2.5 units of Taq DNA polymerase (Amersham Pharmacia Biotech.).

Thermal cycling was carried out with an initial 95°C denaturation step for 10 minutes, followed by 35 cycles of denaturation for 1 minute at 95°C, annealing for 1 minute at 58°C, extension for 1 minute at 72°C and a final extension step at 72°C for 10 minutes. The PCR product was then digested with the restriction enzyme BstU I (Amersham Pharmacia Biotech., USA) for 12 hours at 37°C. The fragment length of the KK genotype was 223 bp; the fragment lengths of the EE genotype were 136 and 87 bp; the fragment lengths of the EK genotype were 223, 136 and 87 bp. The products of the digestion process were separated by electrophoresis on a 2.5% agarose gel, followed by ethidium bromide staining.[4]

Data were statistically described in terms of mean and standard deviation (SD), frequencies (number of cases) and relative frequencies (percentages). Comparison of quantitative variables between the study groups was done using the *t* test and one-way ANOVA test for independent samples. For comparing categorical data, chi-square test was performed. Risk analysis was performed by calculating the odds ratio (OR) and the 95% confidence interval (CI). A *P* value less than .05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

This study included 100 CHD patients, 27 of whom had unstable angina (UA) (22 men and 5 women, mean [SD] age, 54.9 [8.7] years), and 73 patients had acute myocardial infarction (MI) (65 men and 8 women, mean [SD] age, 50.3 [7.9] years). The control group consisted of 50 healthy subjects. There were no significant differences in age and gender between CHD patients and controls. Patients with hypercholesterolemia and smokers were more frequent in the CHD group when compared with controls (**Table 1**).

Distribution of allele and genotype frequencies

The genotype frequencies were 43% EE, 37% EK and 20% KK in patients with CHD, thus differing from those in the control subjects, which were 74% EE, 22% EK and 4% KK. The genotype frequencies were 38.3% EE, 38.3% EK and 23.3% KK in patients with MI and 55.6% EE, 33.3% EK and 11.1% KK in patients with UA, showing no significant difference (**Table 2**).

Table 1. Characteristics of coronary heart disease (CHD) patients (n=100) and controls (n= 50).

Variable	CHD patients	Controls	*P*
Age (years)[a]	52.6 (8.29)	53.9 (6.2)	.34
Gender (male /female)	87 /13	38 /12	.23
Hypertension (%)	71	68	.70
Smoking (%)	65	39	.002
Diabetes mellitus (%)	30	26	.61
Hypercholesterolemia (%)	79	52	.001
ICAM level (pg/mL)[a]	838.79 (153.93)	802.55 (144.68)	.37

[a]Values are mean (standard deviation).

Table 2. Frequencies of the K469E variants of *ICAM-1* gene in CHD (n=100), unstable angina (UA) (n=27), myocardial infarction (MI) (n=73) and controls (n= 50).

	CHD	UA	MI	Controls
ICAM-1 Genotype n (%)				
EE	43 (43)	15 (55.6)	28 (38.33)	37 (74)
EK	37 (37)[a]	9 (33.3)[a]	28 (38.33)[a]	11 (22)
KK	20 (20)[a]	3 (11.1)[a]	17 (23.3)[a]	2 (4.0)
KK+EK	57(57)[a]	12 (44.4)[a]	45 (61.65)[a]	13 (26.0)
ICAM-1 allele n (%)				
E	123 (61.5)	39 (72.2)	84 (57.5)	85 (85)
K	77 (38.5)[a]	15 (27.8)[a]	62 (42.5)[a]	15 (15)

[a]Significantly increased as compared to controls.

Analysis of the genotype with respect to dominant and additive effects of the K allele showed that individuals with K allele (KK and EK) had a significantly increased risk of CHD (**Table 3**).

Serum sICAM-1

sICAM-1 serum levels showed no significant difference between CHD patients (mean [SD], 838.79 [153.93] pg/mL) and control subjects (mean [SD], 802.55 [144.68] pg/mL) (*P*=.37). In the CHD group, sICAM-1 levels were comparable between MI (mean [SD], 828.17 [158.72] pg/mL) and UA (mean [SD], 867.52 [138.87] pg/mL) (*P*=.23). There were no significant differences in sICAM-1 levels between patients with different genotypes; EE (mean [SD], 809.51 [132.8] pg/mL), EK (mean [SD], 816.21 [116.82] pg/mL) and KK (mean [SD], 847.23 [125.7] pg/mL) (*P*=.532). A significant difference was found between men (854.966 [153.33] pg/mL) and women (720.183 [99.36] pg/mL) (*P*=.004).

Table 3. Odds ratios for CHD patients heterozygous or homozygous for the -469 K variant.

Genotype	Odds ratio (95% CI)	*P*
EK	2.9 (1.2-7.8)	.014
KK	8.6 (1.8-57.2)	.003
KK+EK	3.8 (1.7-8.5)	.001

DISCUSSION

Leukocyte adherence to the vascular endothelium is one of the earliest demonstrable events in atherosclerosis.[7] Intercellular adhesion molecule-1 (ICAM-1) mediates the interaction of activated endothelial cells with leukocytes and plays a fundamental role in the pathogenesis of coronary atherosclerosis. ICAM-1 single-base C/T polymorphism, which determines an amino acid substitution from glutamine (E) to lysine (K) in the ICAM-1 protein in exon 6 codon 469, has been described.[8]

In the current study, ICAM-1 EK and KK variants represented 57% of our CHD patients and were associated with increased risk of disease development; OR=3.8 (95% CI: 1.7 to 8.5; ^{P}=.001). This agrees with the findings of Jiang et al in German patients with CHD, who suggested an important role for ICAM-1 gene polymorphism in codon 469 in the susceptibility for CHD and MI. In Han Chinese population, Zhang et al[9] found that the presence of KK genotype of ICAM-1 codon 469 conferred an increased risk for CHD. Also, Liu et al[4] reported that KK homozygotes had a higher risk of re-stenosis after coronary stenting and concluded that the ICAM-1 KK genotype may serve as a predictor of in-stent re-stenosis, especially in obese and hyperlipemia patients. Similarly, Lu et al[10] found that K allele frequency was higher in CHD patients than in controls, and K allele carriers develop myocardial infarction more easily. In Egyptian patients with peripheral arterial occlusive disease, Shaker et al[11] found a statistical difference in the clinical history and laboratory data between the EE and EK variants among those with ischemic heart disease.

A study by Banda et al[12] showed that mice with *ICAM-1* deficiency had normal endothelial function (vasorelaxation in response to acetylcholine) after ischemia-reperfusion, whereas wild-type mice had impaired vasorelaxation in response to acetylcholine, indicating that *ICAM-1* gene function may be related to impaired endothelium-dependent vasodilatation. This dysfunction of the endothelium plays a key role in all stages of atherosclerosis.

On the other hand, McGlinchey et al[13] found no association between the *ICAM-1* K469E polymorphism and CHD in a well-defined Irish population. Similarly, Aminian et al[14] found no significant differences between CHD patients and controls as regards KK genotype and concluded that there was no strong relation between K469E polymorphisms and occurrence of CHD and MI in the studied population from Fars province, Iran. Also, a Slovenian study reported that the K469E polymorphism of the *ICAM-1* gene was not associated with MI in subjects with type 2 diabetes.[15] The discrepancy among the studies could be due to selection of patient groups from different ethnic populations, variable sample sizes or different interactions of the genetic background with environmental factors.

In the present study, the frequencies of the K genotype (KK and EK) in acute MI patients (61.6%) showed no significant difference as compared to UA patients (44.4%) (*P*=.121). Previous studies compared ICAM-1 K469E polymorphisms between MI and CHD group as a whole.[7,14] In our work, there were no significant differences in sICAM-1 levels between CHD patients and control subjects (*P*=.37) and no significant differences in sICAM-1 levels among patients with different genotypes (*P*=.53). However, sICAM-1 level elevation among patients with coronary disease was reported by Ridker et al,[16] Maliket al[17] and Güray et al.[18] Also, in the Han population of China, individuals with the K allele had higher plasma level of sICAM-1 than those without the K allele.[10] This difference in results may be due to the fact that most of the patients in the current study had an acute condition, while the patients in some of the previous studies had chronic CHD. In a prospective study, Albertet al[2] found that among women without a history of cardiovascular disease, sICAM-1 levels were predictive of cardiovascular events that reflect coronary atherosclerotic disease progression and vessel narrowing as stable angina, but not those events associated with acute thrombosis/vessel occlusion as acute myocardial infarction. Discrepancies between sICAM-1 levels in different studies can be attributed to the racial/ ethnic differences in cardiovascular risk.[1]

The present study showed higher levels of sICAM-1 in men than women with CHD (*P*=.004). However, Zakai et al[19] studied the relation between inflammation and cardiovascular disease risk (CVD) in older adults, and they found that sICAM-1 was associated with CVD in women only. This difference may be attributed to the limited number of female patients in our study or the different ages of patients. In comparison between UA patients and acute MI patients, there was no significant difference as regards the sICAM levels (*P*=.23); this agrees with the findings of Gürayet al[18] and Damnjanovic et al.[20]

In conclusion, our results suggest that the KK and EK genotypes of the *ICAM-1* gene polymorphism in codon 469 are associated with the risk for CHD development in Egyptians. Circulating sICAM-1 is not influenced by this polymorphism and is not necessarily elevated in acute coronary heart disease.

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original article

Association of interleukin-1 beta (-511C/T) polymorphisms with osteoporosis in postmenopausal women

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BACKGROUND AND OBJECTIVES: Osteoporosis is a common disease of the elderly, in which genetic and clinical factors contribute to the disease phenotype. Since the production of interleukin-1 (IL-1) has been implicated in the bone mass and skeletal disorders, we investigated whether IL-1 system gene polymorphisms are associated with the pathogenesis of osteoporosis in postmenopausal Taiwanese women.

METHODS: Osteoporosis is diagnosed by dual-energy x-ray absorptiometry, which measures bone mineral density (BMD) at multiple skeletal sites. We studied the IL-1α (-889C/T), IL-1β (-511C/T) and the 86 base pair variable number tandem repeat (VNTR) in intron 2 of the IL-1 receptor antagonist (IL-1ra) gene in 117 postmenopausal women with osteoporosis and 135 control subjects without a history of symptomatic osteoporosis. These gene polymorphisms were analyzed by polymerase chain reaction and restriction fragment length polymerase. Blood sugar and other risk factors were also determined.

RESULTS: The frequencies of IL-1β (-511C/T) genotypes (*P*=.022, odds ratio=1.972) and alleles (*P*=.02, odds ratio=2.909) showed a statistically significant difference between the two groups. However, we did not find any statistically significant difference in IL-1α and IL-1ra polymorphisms (*P*>.05). We also observed a positive relationship between osteoporosis and cholesterol and a weak inverse relationship between blood sugar and osteoporosis in postmenopausal women.

CONCLUSIONS: These experimental results suggest that the pathogenesis of osteoporosis is associated with IL-1β (-511C/T) polymorphism in postmenopausal women. This polymorphism is an independent risk factor for osteoporosis.

Osteoporosis is a common skeletal disorder among the elderly. Symptomatic osteoporosis is due to a reduction in bone mineral density (BMD), which is a risk factor for bone fracture. Low BMD values are strongly associated with osteoporotic fracture. Approximately 50% of all women suffer from osteoporosis; in these women, BMD values fall progressively with age. Several risk factors contribute to osteoporotic fracture, such as advanced age, low body mass index, previous fracture, muscle weakness and a family history of fracture.[1]

A previous study reported that interleukin-1 (IL-1) and IL-1 receptor antagonist (IL-1ra) gene polymorphisms were the most important genetic factors related to bone mass in postmenopausal women.[2] The osteoblast, a relative of the macrophage, is a bone-resorptive cell that contributes to bone formation. The growth of the osteoclast and macrophages are mediated by the IL-1 system.[3] If bone resorption exceeds bone formation, the risk of bone loss and osteoporotic fracture increases. The IL-1 system consists of three proteins: IL-1α and IL-1β are pro-inflammatory proteins, and IL-1ra is an antagonist protein. These proteins are encoded by the genes IL-1α, IL-1β and IL-1ra, respectively.[4] IL-1α and IL-1β are known to be potent stimulators of bone resorption. IL-1ra is a competitive inhibitor of IL-1, as it bonds to the same receptor sites as does IL-1. Both proteins stimulate the proliferation and differentiation of osteoclast precursors into mature osteoclasts.[5,6]

Kim et al showed that there was an association between bone mass and IL-1 system polymorphisms in postmenopausal Korean women.[7] The polymorphisms of IL-1 system, the IL-1α (-889 C/T), IL-1β (-511 C/T) and IL-1ra (86-bp VNTR), have been shown to be strong heritable components,[8-10] but only a few studies have examined the possible relationships between the IL-1 system polymorphisms and osteoporosis, and the results are controversial.[11-14] The relationship between IL-1 system and osteoporosis needs to be elucidated for different populations.

In the study, we hypothesized that IL-1 system polymorphism might be associated with the pathogenesis of osteoporosis. In particular, inheritance of genotype or allele variations in the cytokines genes may predispose to the risk of osteoporosis. To investigate this hypothesis, the polymorphisms of IL-1α (-889 C/T), IL-1β (-511 C/T) and IL-1ra (86 bp VNTR) were analyzed in patients with osteoporosis and in normal controls. Additionally, we examined the association of serum triglycerides and total cholesterol with osteoporosis in postmenopausal women.

METHODS

Postmenopausal women with or without osteoporosis who were not taking hormone therapy were recruited from the Zuoying Armed Forces General Hospital in Taiwan from March to October in 2008. All individuals agreed to participate in the study. Clinical laboratory examination included age, body mass index, plasma glucose, triglycerides and cholesterol. The study plan was accepted and supported by the ethics committee of Zuoying Armed Forces General Hospital. All specimens were collected and stored at −20°C until DNA extraction.

Osteoporosis was defined by a BMD value lower than 2.5 standard deviations below the young adult mean, according to World Health Organization's criteria.[15] BMD was measured by the dual-energy x-ray absorptiometry in grams per centimeter at the lumbar spine and the hip (a total hip or femoral neck).[16] The study excluded all secondary causes of osteoporosis, such as hyperparathyroidism, tumors, or surgical menopausal status.

DNA extraction

Total genomic DNA was extracted with the DNeasyTM kit (Qiagen, USA) according to the manufacturer's instructions. Briefly, the blood was digested with 0.5 mg/mL proteinase K in 400 μL cell-lysis solution for 24 hours at 55°C until the blood was completely lysed. After adding 200 μL absolute ethanol to the lysed sample, the mixture was transferred into the DNeasy mini column and centrifuged for 1 minute at 8000 rpm. The DNeasy mini column was washed with 500 μL washing buffer and centrifuged for 1 minute at 8000 rpm. Finally, the DNA was eluted in a clean 1.5-mL microcentrifuge tube. The amount of DNA was measured spectrophotometrically using a spectrophotometer (GeneQuant) and stored at −20°C until polymerase chain reaction (PCR) amplification.

Determination of IL-1 system gene polymorphisms

The C/T substitution at position -889 in the promoter region was assessed by PCR amplification, and the products were digested. The sequences of PCR primers were 5' GCA TGC CAT CAC ACC TAG TT 3' (sense) and 5' TTA CAT ATG AGC CTT CCA TG 3' (antisense) with an expected PCR product size of 194 bp (from −1062 to −869). Amplification was performed by using a Perkin-Elmer 9700 thermal cycler (Applied Biosystems, Foster City, CA) and polypropylene PCR plates no. 170651 (Biozym, Landgraaf, The Netherlands). The amplification conditions consisted of 94°C for 3 minutes, followed by 45 cycles of 94°C for 1 minute, 56°C for 1 minute and 72°C for 40 seconds. The reaction was terminated by a final elongation at 72°C for 7 minutes. The products were digested with 5 U/μL of NcoI at 37°C for 4 hours and formed 178- and 16-bp DNA products for allele C (allele 1 or wild-type allele) and an intact fragment of 194-bp DNA products for allele T (allele 2 or variant allele). The digested products were separated on a 4% agarose gel and then stained by ethidium bromide (0.5 μg/mL), and genotypes were determined by analyzing different bands. To ensure accuracy, each test was performed three times for each sample. The fragments were to assess the genotypes 1.1, 1.2 and 2.2.

The C/T substitution was located at position -511 in the promoter region of the IL-1β gene. This region was amplified by PCR, using the primers 5' TGG CAT TGA TCT GGT TCA TC 3' (sense) and 5' GTT TAG GAA TCT TCC CAC TT 3' (antisense) (Invitrogen Life Technologies, Breda, The Netherlands) as described previously.[8] Amplification was performed using a Perkin-Elmer 9700 thermal cycler (Applied Biosystems, Foster City, CA) and polypropylene PCR plates no. 170651 (Biozym, Landgraaf, The Netherlands). The following parameters were used: 94°C for 5 minutes, followed by 45 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute and a final incubation at 72°C for 7 minutes followed by a cooling to 4°C. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide. The 305-bp fragments were digested overnight at 37°C with

0.13 U/μL AvaI (New England Biolabs, UK), resulting in fragments that either remained intact (allele 2 or variant allele) or were cut into two fragments of 190 and 114 bp, respectively (allele 1 or wild-type allele). These fragments were analyzed by electrophoresis on 2% agarose gel containing 0.1% ethidium bromide to assess the genotypes 1.1, 1.2 and 2.2.

The polymorphic region, containing 86 base pair variable number of tandem repeats (VNTR) within the intron 2 of the IL-1ra gene, was amplified by PCR with the primers Gloria 1, 5' CTC AGC AAC ACT CCT AT 3' (sense); and Gloria 2, 5' TCC TGG TCT GCA GGT AA 3' (antisense) (Invitrogen Life Technologies).[10] Amplification was performed by using a Perkin-Elmer 9600 thermal cycler (Applied Biosystems) and polypropylene thin-wall tubes no. 179501 (Biozym). The parameters were, an initial denaturation at 94°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute and elongation at 72°C for 1 minute. The final elongation was at 72°C for 5 minutes followed by cooling to 4°C. The PCR products of 412 bp (A1=four repeats of the 86-bp region), 240 bp (A2=two repeats), 498 bp (A3=five repeats), 326 bp (A4=three repeats), 584 bp (A5=six repeats) and 756 bp (A6=eight repeats) were analyzed by electrophoresis on standard 2% agarose gel stained with 0.1% ethidium bromide.

Statistical analysis

Demographic and clinical data were compared between groups by analysis of variance (ANOVA). Genotype and allele frequencies were compared by the chi-square test for small sample size. The *P* values, odds ratios and 95% confidence intervals were calculated. A *P* value of less than .05 was considered significant for all analyses.

RESULTS

We recruited 252 postmenopausal women (aged over 55 years), who were free of chronic diseases and medications known to affect bone formation, including 117 postmenopausal women with osteoporosis, aged between 56 and 74 years (mean age and standard deviation, 65.0 [7.69] years), and 135 postmenopausal women who did not have osteoporosis, aged between 55 and 78 years (mean age and SD, 65.8 [9.49] years) (**Table 1**). Differences in cholesterol and blood glucose levels were significantly different, but there were no significant differences in body mass index and triglycerides.

There was a significant association between the IL-1β (-511C/T) gene and osteoporosis (**Table 2**). Among 117 osteoporosis patients, 30 (25.6%) had the CC genotype compared to 18 (13.3%) of the 135 women without osteoporosis (*P*<.05). Statistically significant differences were found in allele frequency for IL-1β when osteoporosis patients were compared with non-osteoporotic women (*P*<.05). These results indicate that the individuals carrying the genotypic variation of IL-1β (-511C/T) have an increased risk of having osteoporosis.

DISCUSSION

Bone mineralization is associated with differentiation of the osteoblast, which secretes osteopontin, one of the bone matrix proteins involved in bone formation. Rajamannan et al[17] found that expression of osteopontin was stimulated by a high cholesterol diet, since cellular proliferation and bone matrix production were found to be mediated by hypercholesterolemia in in vivo experiments. Kha et al[18] suggested that oxysterols, the products of cholesterol oxidation, contribute to the regulation of stem cell differentiation toward osteoblasts. A study by Brownbill et al[19] showed that higher levels of serum lipids are positively associated with higher bone mineral density in postmenopausal women. Dennison et al[20] demonstrated a relationship between BMD and lipids, but no relationship for BMD with total or LDL cholesterol. However, several studies have reported the opposite relationship, that lower BMD or osteoporosis is associated with higher total cholesterol levels in postmenopausal women.[21,22] We also found a positive relationship between high cholesterol levels and osteoporosis and a weak inverse relationship between blood glucose and osteoporosis, but no relationship between osteoporosis and serum triglycerides and BMI in this study. Similarly, Samelson et al[23] found an inverse relationship between elevated total cholesterol and lower BMD. These contradictory reports indicate that the mechanism of this association between lipid profile and BMD needs to be elucidated. They also lead to the con-

Table 1. Demographic and clinical data for normal and osteoporotic postmenopausal women.

	Osteoporosis (n=117)	Normal (n=135)	*P*
Age (years)	65.0 (7.69)	65.8 (9.49)	NS
Body mass index (kg/m^2)	24.8 (2.71)	26.3 (12.22)	NS
Triglyceride (mg/dL)	110.8 (62.82)	101.0 (78.87)	NS
Cholesterol (mg/dL)	190.2 (22.63)	148.1 (47.67)	.001
Blood glucose (mg/dL)	99.2 (16.01)	120.0 (48.81)	.043

Values are mean (standard deviation)

Table 2. Genotype and allele frequencies of IL-1 system genotypes in non-osteoporotic and osteoporotic postmenopausal women.

Gene	Genotype	Allele	Osteoporosis (n=117)	Normal (n=135)	OR(95% CI)	*P*
IL-1α -889					0.952 (−0.211 - 0.337)	NS
	CC		93 (79.6%)	114 (84.4%)		
	CT		12 (10.2%)	9 (6.7%)		
	TT		12 (10.2%)	12 (8.9%)		
IL-1β -511					1.972 (−0.692 - −0.056)	.022
	CC		30 (25.6%)	18 (13.3%)		
	CT		54 (46.2%)	45 (33.3%)		
	TT		33 (28.2%)	72 (53.4%)		
IL-1ra					0.988 (−0.167 - 0.170)	NS
	A2		21 (17.9%)	24 (17.8%)		
	A1		93 (82.1%)	111 (82.2%)		
IL-1α-889					0.714 (−0.369 - 0.201)	NS
		C	198 (84.6%)	237 (87.7%)		
		T	36 (15.3%)	33 (12.2%)		
IL-1β-511					2.909 (0.042 - 0.472)	.020
		C	114 (48.7%)	81 (30.0%)		
		T	120 (51.2%)	189(70.0%)		

OR: odds ratio, CI: confidence interval

clusion that cholesterol is not a long-term clinical factor contributing to osteoporosis.

Osteoporosis is a common disease of the elderly, suggesting a possible link among various factors, including dietary saturated fat, physical activity, medical treatment and metabolic disorders. Osteoporosis is a polygenic disorder and is affected by the expression of several genes in the regulation of bone formation and osteoporotic fractures; for example, hormones and receptors, such as the vitamin D receptor (VDR) and estrogen receptor (ER); cytokines and receptors, such as IL-1α, IL-1β and IL-1ra, and transforming growth factor β1.[23-27] Among genetic factors in the pathogenesis of osteoporosis, the IL-1 system was found to be the most important cytokine in modulating the growth of bone-resorptive cells in postmenopausal women.[27-30] The IL-1 system is composed of IL-1α, IL-1β and IL-1ra. Both IL-1α and IL-β bond to the IL-1 receptor on the surface of blood cells, and they initiate a cascade of signal transduction to stimulate a potent pro-inflammatory response that initiates bone resorption. IL-1ra also bonds to the same IL-1 receptor, but is a competitive inhibitor of IL-1. However, these results are not consistent in different populations. For example, Langdahl et al[12] reported that osteoporotic fractures were associated with IL-1ra, but not with polymorphism of IL-1β gene in whites. Kim et al[31] reported that the association between IL-1ra VNTR polymorphism and BMD was identified in postmenopausal Korean women. Nevertheless, Bajnok et al[11] observed a lack of association between the IL-1ra gene polymorphism and BMD in postmenopausal Hungarian women. Thus, the association between BMD and IL-1 cytokines is still unclear.[32]

In our study, the allelic and genotypic frequency results showed that IL-1β genotype was more frequent in postmenopausal Taiwanese women with osteoporosis. However, no significant differences were found in the distribution of IL-1α and IL-1ra genotype. This result is consistent with that of previous studies. For instance, Nemetz et al[13] reported that allelic variation at the IL-1β gene was associated with reduction of bone mass in patients with inflammatory bowel disease. Chen et al[14] reported similar findings, that IL-1β and IL-1ra gene polymorphisms were associated with BMD and osteoporosis in postmenopausal women.

In summary, the IL-1β polymorphism was found to be a genetic factor that might influence the maintenance of bone mass in postmenopausal Taiwanese woman. The relationship between IL-1α/ IL-1ra and bone mass is still unclear. Therefore, it is essential to carry out further studies in larger populations and other ethnic groups. Our experimental results suggest that a primary genetic analysis be part of a proper consultation so that precautions can be given to patients on familial genetics.

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Comparing serum basal and follicular fluid levels of anti-Müllerian hormone as a predictor of in vitro fertilization outcomes in patients with and without polycystic ovary syndrome

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BACKGROUND AND OBJECTIVES: The prediction of in vitro fertilization (IVF) outcomes by anti-Müllerian hormone (AMH) measurement is getting increasing attention from clinicians. This study compares the relationship between serum or intrafollicular AMH levels and IVF outcomes in women with and without polycystic ovary syndrome (PCOS).

METHODS: This prospective study was carried out in two university-based fertility clinics. Serum samples were collected on cycle day 3 and follicular fluid (FF) was collected on the day of oocyte retrieval from 26 women with PCOS and 42 normo-ovulatory controls. AMH levels were measured in the samples using immunoenzymatic assay. The relationship between serum or FF AMH levels and IVF outcomes, including number of oocytes retrieved, oocyte maturation rate, fertilization rate, implantation rate, high quality grade embryo rate, and biochemical and clinical pregnancy rates were further assessed.

RESULTS: Median serum basal AMH and FF AMH levels were significantly higher in the PCOS group as compared to controls, the values being 14.2 ng/mL vs. 3.2 ng/mL ($P<.001$) and 8.2 ng/g protein vs. 4.7 ng/g protein ($P<.01$), respectively. In both groups, serum basal AMH levels showed a positive correlation with number of oocytes retrieved ($r=0.323$; $P=.037$ in control vs. $r=0.529$; $P=.005$ in PCOS). In the control group, there was a positive relationship between serum basal AMH levels and percentage of matured oocytes ($r=0.331$; $P=.032$) and implantation rate ($r=0.305$; $P=.05$).

CONCLUSION: Serum basal, and not intrafollicular, AMH levels may be a good predictive factor for quantitative and qualitative IVF outcomes in normo-ovulatory, but not in PCOS patients.

Polycystic ovary syndrome (PCOS) is the most common cause of oligo-anovulation, infertility and hyperandrogenism in women, affecting between 5% and 10% of women of reproductive age worldwide.[1] PCOS is characterized by an excessive number of growing follicles (2- to 3-fold that seen in normal ovaries) up to the stage of 2 to 5 mm in size (small antral follicles).[1] In PCOS patients, the selection of one follicle from this increased pool of selectable follicles and its further maturation to a dominant follicle does not occur. The reason for this last phenomenon is unknown, although inhibition of the local effect of follicle-stimulating hormone (FSH) seems pivotal.[2] In the ovary, anti-Müllerian hormone (AMH) is exclusively produced by the granulosa cells from a wide range of follicles from the primary to the small antral stages of folliculogenesis in the adult rat and human.[3,4] AMH is a glycoprotein of the transforming growth factor-beta (TGF-β) super family, it has become clear that it plays an important role in ovarian function, especially in follicle development and selection.[4,5] AMH seems to inhibit the initiation of human primordial follicle growth[6] and prevents multiple selection of a dominant follicle.[7] In addition, it has been shown that AMH is a negative regulator of follicle growth, acting by reducing the sensitivity of follicles to FSH.[8]

One of the main advantages of AMH measurement in IVF treatment instead of the other markers of ovarian reserve may stem from its low inter- and intra-cycle variability. For this reason, AMH could be used as a menstrual cycle-independent marker of ovarian response to controlled ovarian stimulation.[9] AMH appears to correspond well with antral follicle counts (AFCs) and ovarian response to hyperstimulation in in vitro fertilization (IVF)[10,11] and to be useful for predicting ovarian response in women undergoing IVF treatment.[12] It has been suggested that serum basal AMH levels represent both the quantity and quality of the ovarian follicle pool during the cycle.[13-15] Lack of success in IVF, indicative of a diminished ovarian reserve, is associated with reduced serum basal AMH concentrations.[16-18] In PCOS, the increase in serum basal AMH level has been shown to be closely related to the degree of menstrual disorder[19] and with the excess in the 2 to 5 mm follicle number (FN) on ultrasonography (US).[20,21] The role of AMH as a predictive factor of IVF outcomes remains a contentious subject in patients with PCOS. This study sought to determine the relationship between serum basal and intrafollicular AMH levels with the IVF-embryo transfer (ET) outcomes, including number of oocytes retrieved, oocyte maturation rate, fertilization rate, implantation rate, percentage of high quality grade embryo, and biochemical and clinical pregnancy rates in patients with and without PCOS.

METHODS

This prospective study included 26 infertile women with PCOS, 21 to 37 years of age and with body mass index (BMI) ranging between 17 kg/m2 and 32 kg/m^2. Infertility due to reduced sperm count or abnormality of sperm was excluded in this group. According to the Rotterdam criteria,[22] the diagnosis of PCOS was based on the association of at least two of the following three criteria: 1) ovulatory disturbance, mainly oligomenorrhea or amenorrhea; 2) hyperandrogenism, as defined either by hirsutism, seborrhea, and/or testosterone > 0.7 ng/mL and/or androstenedione > 2.2 ng/mL, as measured on day 3 of the cycle; 3) more than 12 follicles in the 2- to 9-mm range in each ovary at US and/or ovarian volume higher than 10 mL. The study was approved by the Institutional Review Board of the Vali-e-Asr Reproductive Health Research Center and Shariati University Hospital of Tehran.

The control group comprised 42 infertile normo-ovulatory women 24 to 42 years of age. All met the following inclusion criteria: 1) both ovaries were present; 2) menstrual cycle length was between 25 and 35 days; 3) there were no current or past diseases affecting the ovaries or gonadotropin or sex steroid secretion; 4) there were no clinical signs of hyperandrogenism; 5) FSH levels were $\leq$10 mIU/mL on day 3 of the cycle; 6) BMI ranged between 21 kg/m^2 and 35 kg/m^2. In all cases, infertility was due to sperm or tubal abnormalities, endometriosis, or was unexplained.

The IVF/ET treatment was initiated with the oral contraceptive pill (OCP) on day 3 or 5 of the cycle and continued with pituitary downregulation by a gonadotropin-releasing hormone (GnRH) agonist consisting of 0.5 mg of buserelin (Suprefact, 1mg/cc, Aventis, Germany) in the mid-luteal phase (day 21) of the cycle, given daily until the day of human chorionic gonadotropin (hCG) administration. On day 3 of the new cycle, ovarian stimulation was started with an injection of recombinant FSH (Gonal-F 75 IU, Serono, Switzerland) or human menopausal gonadotropin (hMG) (Menopur 75 IU FSH: 75 IU LH, Ferring, Germany). The gonadotropin dosage was decided according to the follicular growth, which was monitored by US. The patients were given 10 000 IU of hCG (Pregnyl, Organon, Iran) when at least three follicles became >17 mm. The oocytes were picked 34 h after hCG administration and IVF/ICSI followed by embryo transfer was performed 2 days after oocyte pick-up.

On day 3, the women underwent blood sampling. Sera was separated and frozen in aliquots at −20°C for subsequent analysis. On the day of oocyte pick-up, the FF from more than one follicle (16-20 mm) was gently aspirated and subsequently frozen at −20°C for AMH measurement using an ultrasensitive enzyme-linked immunosorbent assay (Immunotech Coulter, Marseilles, France). The lower detection limit was estimated at 0.1 ng/mL, corresponding to 0.7 pmol/L. The intra- and interassay coefficients of variation were 12.3% and 14.2%, respectively. To avoid possible bias due to FF volume variability, AMH concentrations in the FF were adjusted to its protein content by the Bradford assay. Briefly, a 5× stock of Bradford reagent was prepared as follows: 0.05% (w/v) Bradford reagent + 25% ethanol (v/v) + 42.5% phosphoric acid were mixed together with constant shaking for 2-3 h at room temperature and filtered through Whatman filter paper. All the reagents were provided by Merck and Co. Inc, USA Company. Successive dilutions of BSA (mg/mL) were prepared from a stock solution of BSA 1 mg/mL (Sigma-Aldrich Co, UK) for determination of the standard curve. After 900 μL of 1× Bradford reagent was added to 100 µL diluted FF samples (1/2000), the absorbance of standard and unknown solutions were determined at 595 wavelength using a spectrophotometer (Biorad-Laboratories, Inc. UK). Protein concentration was determined by using linear regression (Microsoft Excel) and the results were expressed as nanogram per gram (ng/g) of protein.

To determine differences between groups, the Mann-Whitney U test was used and correlations were expressed as Spearman correlation coefficients. Multiple logistic regression analysis was used to study independent association among a set of variables. *P*≤.05 was considered to be statistically significant. All statistical procedures were run on SPSS version 13 (SPSS Inc, Chicago, IL).

Table 1. Clinical data in control and PCOS group.

	Control (n=42)	PCOS (n=26)	*P*
BMI (kg/m^2)	27.0 (21-35)	27.0 (17-32)	.370
Oocyte number	9.0 (3-22)	11.0 (2-26)	.066
Oocyte maturation (%)	78.0 (25-100)	71.0 (31-100)	.095
Number of transferred embryos	3.0 (1-5)	3.0 (0-5)	.648
High-quality grade embryo (%)	88.5 (38-100)	82.4 (0-100)	.311
Fertilization rate (%)	62.3 (14-100)	75.5 (50-100)	.128
Implantation rate (%)	5.02	9.19	.265

Values are expressed as median (range) for clinical data. *P*≤.05 was considered significant.

Table 2. Relationship between AMH levels in serum (ng/mL) and FF (ng/g protein) with IVF/ET outcomes.

	Control (n=42)		PCOS (n=26)	
	r	*P*	r	*P*
Serum AMH, fertilization rate	−0.247	0.115	0.053	.796
Serum AMH, implantation rate	0.305	0.050[a]	−0.299	.138
Serum AMH, high quality grade embryo	−0.108	0.494	0.150	.474
FF AMH, fertilization rate	−0.035	0.827	−0.111	.589
FF AMH, implantation rate	0.246	0.117	0.134	.513
FF AMH, high quality grade embryo	0.091	0.568	−0.309	.133
FF AMH, oocyte number	−0.118	0.456	−0.162	.429
FF AMH, oocyte maturation	0.275	0.078	0.186	.363

P≤.05 was considered significant. [a]*P*≤.05.

RESULTS

In the control group, we found an inverse relationship between serum basal AMH levels and age (r =−0.0323, *P*=.037) and BMI (r =−0.321, *P*=.050), but there was no such a correlation in the PCOS group. Intrafollicular AMH concentration remained unaffected by age and BMI in both groups (data not shown). The main clinical data in the control and PCOS groups are presented in **Table 1**. There were no statistically significant differences between the control and the PCOS group in BMI, number of oocytes retrieved, oocyte maturation rate, percentage of high quality grade embryos, and fertilization or implantation rates. Although, biochemical and clinical pregnancy rates were higher in the PCOS group compared to controls, the difference was not statistically significant.

Median range serum AMH levels were significantly increased in the PCOS group (14.2 ng/mL [0.64-50.7] vs. 3.2 ng/mL [0.42-9.90]; *P*<.001) (**Figure 1a**). Similarly, median FF AMH levels were higher in the PCOS group (8.2 ng/g protein [1.6-141.0] vs. 4.7 [0.4-120.0]; *P*=.004) (**Figure 1b**). As shown in **Figure 2**, serum basal AMH levels were positively and significantly correlated with number of oocytes retrieved in both groups (r=0.323; *P*=.037 in control vs. r=0.529; *P*=.005 in PCOS). Similarly, a positive and significant relationship between serum basal AMH levels and oocyte maturation rate (number of matured oocytes×100/number of oocytes retrieved) was observed in control group (r=0.331; *P*<.05), as revealed in **Figure 3**. However, intrafollicular AMH levels remained unaffected by oocyte quantity and quality. The relationship between serum basal and intrafollicular AMH levels with IVF/ET outcomes for both groups is shown in **Table 2**. Fertilization rate (number of embryos forming×100/injected oocyte number) was unrelated to serum basal and intrafollicular AMH levels in both groups. In the control group, the implantation rate (total number of gestation sacs×100/total number of embryo transferred) showed a positive and significant relationship with serum basal AMH level. Day 2 cleavage-stage embryos were graded 1-4 by the embryologist according to established morphological criteria, with good quality embryos (grades 1-2) being preferentially transferred rather than those of poorer quality (grades 3-4). High-quality grade embryo (number of the embryos forming (grade 1 + grade 2) ×100/total number of embryo)remained unaffected by both serum basal and intrafollicular AMH levels in both groups.

The serum basal AMH level, but not intrafollicular AMH level-showed a positive correlation (*P*=.054) with biochemical pregnancy rate in the control but

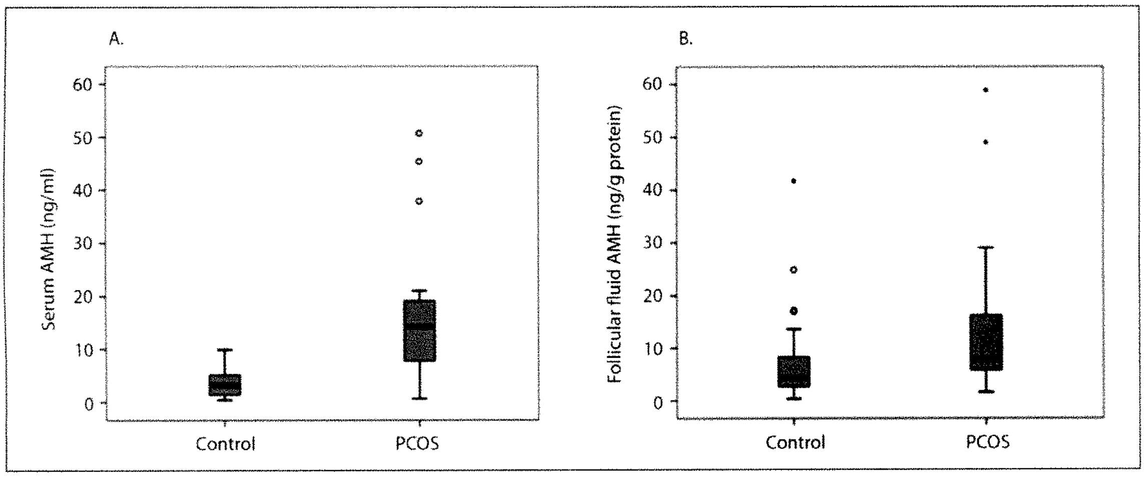

Figure 1. Box plots showing the values of AMH in A) serum (ng/mL) and B) FF (ng/g protein) in control and PCOS groups. Serum and FF AMH levels were significantly higher in the PCOS group than in controls, with *P*<.001 and *P*<.01, respectively.

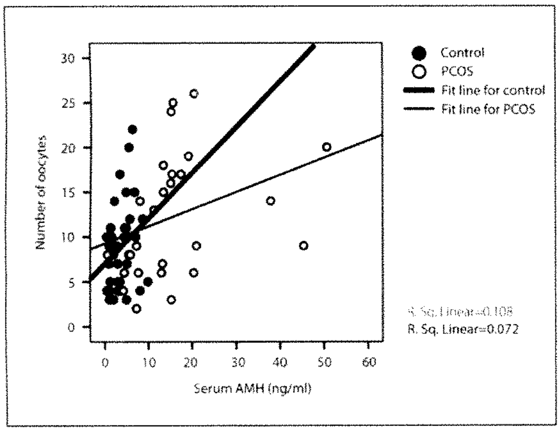

Figure 2. Correlation of serum AMH with oocyte number (r=0.323; *P*<.05 in control vs. r= 0.529; *P*<.01 in PCOS) in control and PCOS groups.

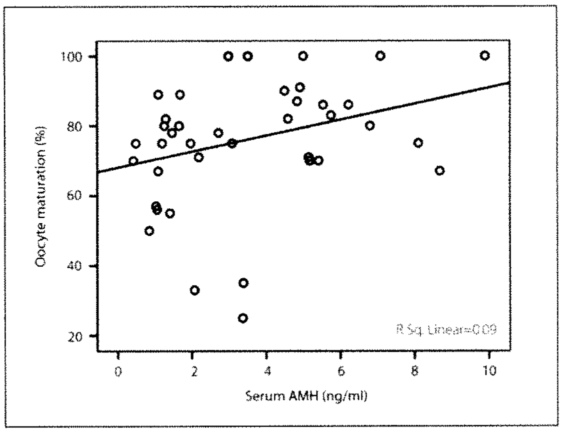

Figure 3. Correlation of serum AMH with oocyte maturation (r=0.331; *P*<.05) in control group.

not in the PCOS group. No significant correlation was observed between serum or intrafollicular AMH levels and clinical pregnancy rate (gestational sac as observed at US after 7 weeks of amenorrhea) in both groups. Moreover, when assessed by multiple logistic regression analysis, none of the assessed outcomes had a significant effect on biochemical/clinical pregnancy rates.

DISCUSSION

The aim of this study was to compare serum basal to intrafollicular AMH to assess their usefulness for predicting quantitative and qualitative IVF outcomes in patients with and without PCOS. In summary, the results of this study clearly indicate that serum basal and intrafollicular AMH levels are significantly higher in women with PCOS than in controls. Furthermore, the results suggest that serum basal AMH level is predictive of oocyte quantity and quality, as is evident by the higher oocyte maturation rate in control than in PCOS patients. On the other hand, intrafollicular AMH level could be a good marker for oocyte quantity but not their quality. So far as is known, this study is the first to describe a significant link between serum basal AMH levels and implantation rate in normo-ovulatory women. These results strengthen the body of evidence supporting the use of day 3 serum AMH measurements for the prediction of pregnancy outcomes during IVF treatment in normal control but not in PCOS patients.

AMH appears to correspond well with antral follicle counts (AFCs) and ovarian response to hyperstimulation in IVF.[10,11,23] The increased serum basal AMH concentrations in PCOS have been explained by the increased number of small ovarian follicles responsible for AMH secretion.[20] We also demonstrated that FF AMH levels are greater in women with PCOS compared to normo-ovulatory women, which may suggest increased per follicle AMH secretion in PCOS.[24]

All the studies (including this report) have confirmed the utility of early follicular phase serum AMH concentration as a predictor of the number of retrieved oocytes, which could support the concept of a direct relation between serum AMH levels and AFCs in an IVF cycle.[9,10,15,18,19,24-26] However, data on serum or FF AMH levels in relation to oocyte quality are still contradictory. Like our report, one study demonstrated a positive and significant correlation between serum basal AMH levels and proportion of oocyte in metaphase II.[27] On the other hand, a recent study showed no correlation between serum day 3 AMH levels and oocyte maturation.[28] Ebner showed that serum basal AMH values between 1.66 and 4.52 ng/mL were associated with high-quality oocytes, but higher serum AMH levels could be harmful for oocyte maturation.[16] Moreover, serum AMH levels at ovulation triggering day were more closely related with immature rather than mature oocyte proportion.[29]

We have shown here a nonsignificant inverse relationship between intrafollicular AMH levels and oocyte quantity in both PCOS and control groups. It has been postulated that AMH decreases the sensitivity of growing follicles to FSH and final selection of dominant follicles.[8,30] Thus, it is tempting to speculate that high intrafollicular AMH levels may have a negative effect on oocyte quantity. Until now, the relationship of FF AMH levels with oocyte quality was uncertain. Cupisti et al found that AMH levels in individual follicles were inversely correlated with the maturation and developmental potential of oocytes.[31] On the contrary, Takahashi et al observed that oocytes were more likely to be fertilized when their follicle was able to produce high levels of AMH, as FF AMH levels from follicles with fertilized oocytes were more than three times higher than from follicles with non-fertilized eggs in normo-ovulatory women.[32] However, Mashiach et al demonstrated that in PCOS patients, the proportion of matured oocytes, as well as the success of fertilization, did not correlate with FF AMH.[33] Indeed, excess in FF AMH was shown to have harmful consequences on oocyte quality and final maturation.[34] On the whole, it seems that determination of serum or intrafollicular AMH cutoff values could be of great importance for understanding the relationship with oocyte quality.

Most studies (including this report) have shown that fertilization rate, high quality grade embryo, and clinical pregnancy rates are unaffected by AMH levels.[32,35-38,21,25] In addition, trying to identify cutoff values for serum basal and FF AMH levels, Fanchin et al showed that intrafollicular AMH level is a better predictor of implantation and clinical pregnancy rate than serum basal AMH levels in normo-ovulatory patients; however, this was not confirmed by other studies.[35] We have shown a correlation between serum basal AMH levels and implantation rate in normo-ovulatory patients, which needs to be further examined in a larger study.

In conclusion, serum basal AMH levels could be a good marker for oocyte number in both PCOS and infertile normo-ovulatory women. Further, we suggest that serum AMH level may be a predictive marker of oocyte maturation and implantation rate in normal women. The lack of consistency between AMH levels and qualitative IVF outcomes in PCOS patients may be influenced by the presence of differing PCOS phenotypes, with either primary ovarian dysfunction or insulin and obesity being the major contributor to reproductive dysfunction.

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original article

The pattern of skin diseases in the Qassim region of Saudi Arabia: What the primary care physician should know

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BACKGROUND AND OBJECTIVES: Epidemiological studies to determine the burden of skin diseases are important for proper health care planning. The purpose of this study was to find the pattern of skin diseases in our patients attending university-affiliated dermatologic clinics in the Qassim region.

METHODS: We conducted a prospective study of all Saudi patients attending the Qassim University Medical College-affiliated dermatology clinics of the Ministry of Health for a period of 12 months from 1 March 2008 to 28 February 2009.

RESULTS: The study included 3051 patients comprising 1786 (58.5%) males and 1265 (41.5%) females. Males outnumbered females (P<.05) (male-to-female ratio, 1.4:1). The mean age (standard error of the mean) of the patients was 25.3 (0.27) years. About 71% of the patients were between 5 and 34 years of age. The top five skin diseases were eczema/ dermatitis (19.5%), viral infections (16.6%), pilosebaceous disorders (14.4%), pigmentary lesions (11.2%) and hair disorders (7.6%). The major disorder in males was viral skin infections (20.0%), while eczema/dermatitis (20.7%) constituted the most prevalent skin disease in females. Seasonal variations were recorded in cases of pigmentary lesions, papulosquamous disorders and protozoal infections.

CONCLUSION: Infectious skin diseases, eczema/dermatitis, pilosebaceous disorders, pigmentary lesions and hair disorders ranked as the top five skin diseases. Appropriate training programs for diagnosing and managing common skin diseases should be initiated for primary health care physicians and other general practitioners so as to decrease referrals to dermatology clinics.

Skin diseases are among the common skin problems that are encountered by primary care physicians. The prevalence of skin diseases in any region or country depends on various factors, such as genetics, racial constitution, social and hygienic standards, customs, nutritional status and climatic conditions. In addition, the diagnostic competence of doctors, expertise of dermatologists and availability of the latest diagnostic facilities play a crucial role.[1]

Every individual suffers from skin disease at some point in time during his lifetime. Conditions such as warts and acne are almost universal at certain ages.[2] However, whether people recognize or report these common conditions varies according to the area affected and the severity of the problem. Epidemiological surveys to determine the burden of dermatological disorders in an area are important. The information gathered about the prevalence and spectrum of specific disorders helps in the development of strategies for proper health care planning. Such information also helps to establish proper preventive and research programs according to the need of the community.

The literature on the patterns of general and specific skin diseases is scanty, and only a few published reports are available on Saudi Arabia.[3] Though community-based studies are the best to determine the incidence of a particular disease, they are difficult to carry out. As such, most of the studies to determine the incidence or prevalence of dermatological diseases are based upon hospital attendees.[4,5]

The Qassim region of Saudi Arabia is undergoing rapid development and urbanization, which may be affecting the spectrum of skin diseases in the area. No epidemiological survey is available to estimate the prevalence rate of various skin diseases in the area. The purpose of this study was to find out the pattern of skin diseases in our patients attending the university-affiliated dermatologic clinics in the Qassim region so that appropriate training programs on common skin diseases could be initiated for primary health care physicians and other general practitioners.

METHODS

This prospective study was conducted in the Qassim University Medical College-affiliated dermatology clinics of the Ministry of Health, Qassim region, for a period of 12 months from 1 March 2008 to 28 February 2009. The Qassim region is located in the central part of the Arabian Peninsula, with a population of 1.08 million. It has hot and dry summers, cold winters and rainfall in April and May. The region has an agricultural belt with a sizeable farming community. Approval for conducting this study was obtained from the research and ethical committee of Qassim College of Medicine. Only Saudi patients who were permanent and original residents of the Qassim region were included in the study. Patients who were non-Saudis or of other ethnicities or expatriates were excluded from the study to avoid any confounding factors. All new and old patients attending a dermatology clinic for skin problems during this period were included in this study. All patients were seen by a consultant dermatologist. The diagnoses were mainly based on clinical examination, but were supported by laboratory investigations and histopathology wherever needed. Clinical data on age, sex and diagnosis were collected. All data were processed and analyzed using the Statistical Package for Social Sciences (SPSS version 14). Statistical significance was determined by the (2 and *t* tests. The level of significance was set at $P<.05$.

RESULTS

Of 3051 patients with skin diseases seen during the study period, there were 1786 (58.5%) males and 1265 (41.5%) females. The male-to-female ratio was 1.4:1, and the difference was statistically significant ($P<.05$). The mean (SD) age of our patients was 25.3 (14.9) years. A statistically significant difference was seen in the mean (SD) age of males (26.0 [10.1]) as compared to that of females (24.4 [13.9]; $P=.001$). The distribution of males and females into various age groups is shown in **Figure 1**. Female patients outnumbered male patients younger than the age 24 years, but after that the number of male patients outgrew that of the female patients. **Figure 1** also shows a clustering of patients at younger ages. About 71% of our patients were between 5 and 34 years of age, with a maximum number of patients presenting between ages 15 and 24 years (37.7% females and 34.7% males).

Figure 2 shows the frequencies of various skin disorders in order of ranking. Of the total percentage of eczema/dermatitis cases (19.5%), atopic dermatitis and housewife dermatitis constituted 7.4% and 4.3% of those cases, respectively (**Table 1**). Of patients with viral infections (16.6%), viral warts were found in 13.2% of the viral infection cases. Among the pilosebaceous disorders (14.4%), acne vulgaris (13.5%) was the predominant condition. Among pigmentary disorders (11.2%), vitiligo, post-inflammatory hyperpigmentation and melasma were present in 5.2%, 3.7% and 1.9% of the patients, respectively. Diffuse hair loss and alopecia areata were present in 3.9% and 2.7% of the patients, respectively. Infections of all types accounted for 28.5% of skin diseases.

The prevalence of different skin diseases differed between males and females. In males, viral infections were the most prevalent skin disorder, while in females eczema/dermatitis were the most prevalent. The prevalence rates of viral infections, papulosquamous disorders, bacterial infections and protozoal infection were significantly higher in males as compared to females, while prevalence rates of pilosebaceous disorders, pigmentary disorders and hair disorders were significantly higher in females as compared to males.

The spectrum of diseases varied in different age groups (**Table 2**). The eczema/dermatitis were pre-

Table 1. Comparison of disease pattern between genders (n= 3051).

Disease	Male (n=1786)	Female (n=1265)	*P*
Eczema/dermatitis	333 (18.6)	262 (20.7)	.145
Viral infections	358 (20.0)	149 (11.8)	.001
Pilosebaceous disorders	210 (11.8)	230 (18.2)	.001
Pigmentary disorders	149 (8.3)	192 (15.2)	.001
Hair disorders	104 (5.8)	127 (10.0)	.001
Papulosquamous disorders	119 (6.7)	39 (3.1)	.001
Urticaria	83 (4.6)	71 (5.6)	.203
Fungal infections	99 (5.5)	52 (4.1)	.083
Bacterial infections	83 (4.6)	37 (2.9)	.019
Protozoal infection	88 (4.9)	4 (0.3)	.001
Keratinization disorders	24 (1.3)	18 (1.4)	.749
All others	136 (7.6)	84 (6.6)	.267

Values are number (percent).

Table 2. Distribution of skin diseases by age groups (n= 3051).

Diseases	Total	Age groups						
		<5 years (n=158) No. (%)	5-14 years (n=502) No. (%)	15-24 years (n=1097) No. (%)	25-34 years (n=577) No. (%)	35-44 years (n=342) No. (%)	45-54 years (n=224) No. (%)	55+ years (n=151) No. (%)
Eczema/dermatitis	595	86 (54.4)	120 (23.9)	152 (13.9)	84 (14.6)	58 (17.0)	49 (21.9)	46 (30.5)
Viral infection	507	15 (9.5)	118 (23.5)	172 (15.7)	96 (16.6)	66 (19.3)	24 (10.7)	16 (10.6)
Pilosebaceous disorders	440	2 (1.3)	25 (5.0)	321 (29.3)	70 (12.1)	9 (2.6)	9 (4.0)	4 (2.6)
Pigmentary disorders	341	6 (3.8)	60 (12.0)	134 (12.2)	58 (10.1)	43 (12.6)	31 (13.8)	9 (6.0)
Hair disorders	231	6 (3.8)	32 (6.4)	91 (8.3)	66 (11.4)	25 (7.3)	9 (4.0)	2 (1.3)
Papulosquamous disorders	158	7 (4.4)	41 (8.2)	40 (3.6)	27 (4.7)	16 (4.7)	16 (7.1)	11 (7.3)
Urticaria	154	1 (0.6)	13 (2.6)	30 (2.7)	40 (6.9)	23 (6.7)	21 (9.4)	26 (17.2)
Fungal infection	151	9 (5.7)	36 (7.2)	43 (3.9)	22 (3.8)	19 (5.6)	13 (5.8)	9 (6.0)
Bacterial infection	120	11 (7.0)	18 (3.6)	36 (3.3)	26 (4.5)	13 (3.8)	12 (5.4)	4 (2.6)
Protozoal infection	92	5 (3.2)	2 (0.4)	17 (1.5)	27 (4.7)	30 (8.8)	9 (4.0)	2 (1.3)
Keratinization disorders	42	0	14 (2.8)	14 (1.3)	5 (0.9)	3 (0.9)	4 (1.8)	2 (1.3)
All other	220	10 (6.3)	23 (4.6)	47 (4.3)	56 (9.7)	37 (10.8)	27 (12.1)	20 (13.2)

Values are number (percent).

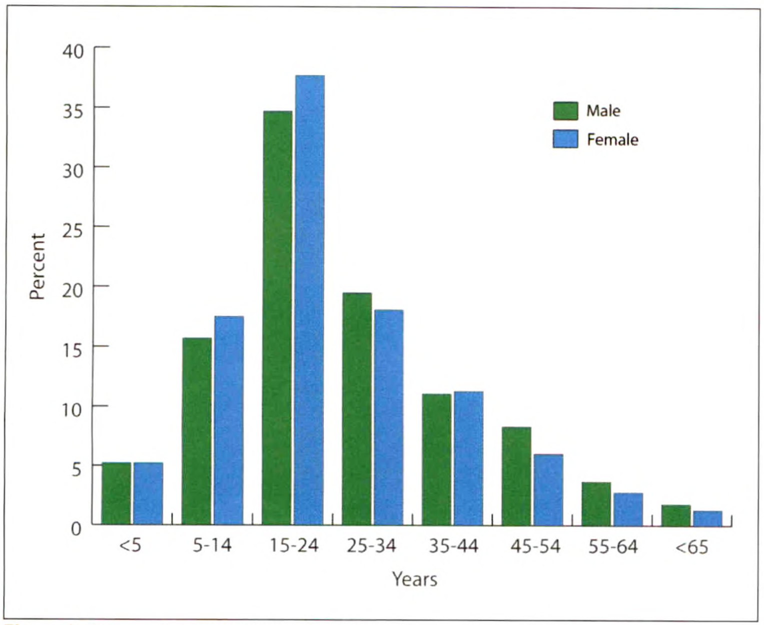

Figure 1. Patient distribution by age and sex.

dominant in the following age groups: <5 years (54.4%), 5-14 years (23.9%), 45-54 years (21.9%) and 55 years and above (30.5%). Viral skin infections were the most prevalent disorder affecting age groups 5-14 years (23.5%), 25-34 years (16.6%) and 35-44 years (19.3%). The predominant skin problem at 15-24 years of age was pilosebaceous disorders (29.3%). The prevalence of pigmentary disorders was significantly lesser in children under 5 years (3.8%) and older patients of age 55 years and above (6%). The prevalence of urticaria, on the other hand, was significantly higher in the older age group of 55 years and above (17.2%).

Pigmentary disorders and papulosquamous disorders occured more frequently in winter and spring (12.4% and 5.9%, respectively) as compared to summer and autumn (9.6% and 4.3%, respectively) (**Table 3**). A significantly higher occurrence of protozoal infections was recorded in summer and autumn as compared to winter and spring (4.3% vs. 2%; $P<.05$). Seasonal variation was not significant in other diseases.

DISCUSSION

Many epidemiological studies around the world, including a few from Saudi Arabia, have claimed that female patients predominate at dermatology clinics due to the greater sensitivity of women to health-related issues.[6-8] In contrast, our study indicated that male patients attended dermatology clinics more frequently. This finding is also supported by many other studies from Saudi Arabia.[2,5,9] The higher prevalence of male patients at dermatology clinics may be attributed to under-representation of females at Saudi clinics due to cultural barriers. Our results also indicated a trend for female patients to outnumber male patients between ages 5 and 24 years. After this age, the number of male patients was greater than that of female patients (**Figure 1**). This observation indicates that women at younger ages are more conscious of their body image, but lose interest as they grow older. Another explanation for this phenomenon could be the preponderance of female patients with acne presenting around this age (**Table 2**).

The major bulk of our patients (71%) presented between ages 5 and 35 years. The major disease burdens were eczema/dermatitis, pilosebaceous disorders and viral infections, which occurred with greater frequency around this age (**Table 2**). The prevalence of various skin diseases varies according to geographical area and is related to racial, environmental and socioeconomic factors of the population. Eczema and dermatitis are the most prevalent skin disorders reported from developed countries, whereas skin infections are predominant in developing African and Asian countries.[6,10] In a recent study from Tunis, Souissi et al reported 38.6% rate of skin infections in a series of 28 224 patients. Fungal infections were the most frequently seen skin infection (43.8%) in their study.[11] Similar results have been reported from Iran by Baghestani et al. Infectious and parasitic diseases, including infections of the skin and subcutaneous tissues, were found to be the most common skin diseases (32.1%) in their study.[7] A review of literature has indicated that bacterial infections tend to decrease in frequency with improved quality of life and better hygienic conditions,[11] whereas advanced industrialization results in a high prevalence of industrial dermatitis and allergic contact dermatitis.[10]

The overall prevalence of skin infections, including viral, fungal, bacterial and protozoal infections, was 28.5% in our study. Most skin infections were viral. Our results have shown that infections, eczema/dermatitis, pilosebaceous disorders, pigmentary lesions and hair disorders ranked among the top 5 skin diseases (**Figure 2**). The results of this study are comparable to those of other studies from Riyadh, Abha, Jouf, Hail, Jeddah, Najran, the Eastern Province and the Asir region of Saudi Arabia.[2,3,5,8,9,12,13] These studies have reported a similar pattern of skin diseases in Saudi patients.

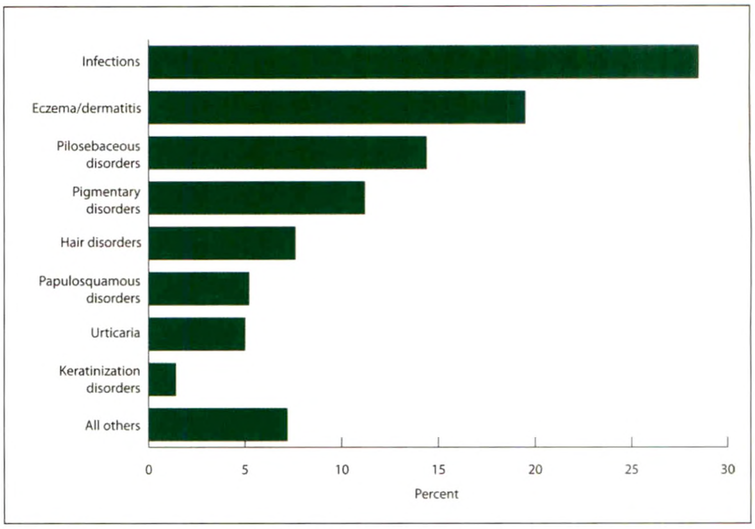

Figure 2. Overall ranking of skin disease.

Table 3. Seasonal variation of skin diseases.

Disease	Total	Season		*P*
		Winter and spring (n=1691)	Summer and autumn (n=1360)	
Eczema/dermatitis	595	333 (19.7)	262 (19.3)	.767
Viral infection	507	289 (17.1)	218 (16.0)	.434
Pilosebaceous disorders	440	227 (13.4)	213 (15.7)	.071
Pigmentary disorders	341	210 (12.4)	131 (9.6)	.015[a]
Hair disorders	231	127 (7.5)	104 (7.6)	.947
Papulosquamous disorders	158	100 (5.9)	58 (4.3)	.041[a]
Urticaria	154	89 (5.3)	65 (4.8)	.497
Fungal infection	151	85 (5.0)	66 (4.8)	.753
Bacterial infection	120	77 (4.6)	43 (3.2)	.053
Protozoal infection	92	34 (2.0)	58 (4.3)	.001[a]
Keratinization disorders	42	25 (1.5)	17 (1.2)	.471
All other	220	95 (5.6)	125 (9.2)	.001[a]

Values are number (percent).

Seasonal variations in certain skin disorders are a well-known phenomenon that has been observed for centuries. A change in temperature, humidity, ultraviolet rays, wind and atmospheric pollen allergens occurs with changing seasons. Low temperature and humidity can have a detrimental effect on the epidermal barrier.[14] Similarly, warm and humid environmental conditions are conducive to the development of fungal infections.[15] Hancox et al studied the seasonal variation of dermatological diseases in the USA, and their results confirmed a significant seasonality for skin diseases (P=.002). The prevalence of actinic keratosis was significantly higher in spring, while acne and folliculitis were significantly more prevalent in winter. They also noted a trend towards seasonality in cases of dyschromia, seborrheic keratosis, seborrheic dermatitis, and psoriasis. Spring was the peak season for dyschromia, seborrheic keratosis and seborrheic dermatitis, while psoriasis was more prevalent in winter.[16]

When comparing the climate in our study setting with the climate in the settings of the studies conducted in Saudi Arabia, we observed that Riyadh, Jouf, Hail were similar, while the Asir region had a moderate climate and was rainy at most times of the year and had higher humidity levels than Qassim, whereas Jeddah and the eastern region have hot humid summers and moderately cold winters.[17] The results of our study support some of the observations made in the study by Hancox et al. We found a significantly higher rate of pigmentary disorders and papulosquamous disorders during spring and winter (P=.015 and .041, respectively). We hypothesize that the possible reason for an increased prevalence of pigmentary disorders during winter months may be an erroneous belief among people that the role of the sun in affecting the skin is only during the time when the sunrays are hot (summer), so during winter (as the rays are not causing any feeling of hotness on the skin) most people do not follow protective measures to avoid the harmful effects of the sun. Protozoal infections were significantly more frequent in summer and autumn (P<.001). Seasonal variation was not significant in other diseases (**Table 3**).

This is the first study to describe the pattern of skin diseases in the Qassim region of Saudi Arabia. Because this study was conducted at Qassim University Medical College-affiliated dermatology clinics of the Ministry of Health, our results provide a rough estimate of the incidence of skin diseases in the Qassim region. However, due to the larger number and homogeneity of our study subjects, we believe that our results may actually mimic the pattern of skin diseases prevalent in the general population of the Qassim area. However, we recommend a large-scale population-based epidemiological study to estimate the true pattern of prevalent skin diseases.

In conclusion, infections, eczema/dermatitis, pilosebaceous disorders, pigmentary lesions and hair disorders ranked among the top five skin diseases. All primary health care physicians and general practitioners should be able to diagnose and manage these common skin disorders. All the cases that were seen in the university-affiliated dermatology clinics of the Ministry of Health were referred from primary health care clinics. We recommend that a series of training programs for diagnosing and managing common skin disorders be initiated for primary health care physicians and other general practitioners. This will result in a decrease in referrals to the dermatology clinics and provide cost-effective and efficient dermatology-related health services at the primary health care level. This study gives us a fair picture of the conditions on which the training of primary health care physicians should be focused. These conditions are skin infections (mainly viral), atopic dermatitis, contact dermatitis, acne vulgaris, vitiligo, post-inflammatory hyperpigmentation, melasma, alopecia areata and diffuse hair loss.

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Glycemic changes after vitamin D supplementation in patients with type 1 diabetes mellitus and vitamin D deficiency

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BACKGROUND: A prospective, nonblinded and nonrandomized controlled trial was conducted to test the hypothesis that vitamin D supplementation would improve glycemic control in patients with type 1 diabetes mellitus who have vitamin D deficiency.

PATIENTS AND METHODS: Eighty patients with type 1 diabetes mellitus who had 25-hydroxyvitamin D levels less than 50 nmol/L were assigned to receive 4000 IU of vitamin D3. Calcium supplements were provided to ensure a total calcium intake of 1200 mg/d. Glycosylated hemoglobin and 25-hydroxyvitamin D levels were measured at baseline and at 12 weeks.

RESULTS: There was a significant difference in mean (SD) glycosylated hemoglobin level (%) between the groups that achieved 25-hydroxyvitamin D levels of <35.4 nmol/L, 35.4-51 nmol/L and >51 nmol/L at 12 weeks (*P*=.02). There was a significant difference in glycosylated hemoglobin change from baseline between the groups that achieved 25-hydroxyvitamin D levels of <35.4 nmol/L, 35.4-51 nmol/L and >51 nmol/L at 12 weeks (*P*=.04). There was a significant difference in 25-hydroxyvitamin D level between the groups that achieved glycosylated hemoglobin levels of <7.8, 7.8-9.9 and >9.9 at 12 weeks (*P*=.001). Patients were more likely to achieve lower glycosylated hemoglobin levels at 12 weeks if they had higher 25-hydroxyvitamin D levels at 12 weeks (r=–0.4, *P*=.001).

CONCLUSIONS: There was an observed effect of vitamin D supplementation on glycemic control in vitamin D-replete, type 1 diabetes mellitus patients. Further studies are needed to determine if these findings are applicable.

Type 1 diabetes mellitus results from cellular-mediated autoimmune destruction of the beta cells of the pancreas.[1] Serum 25-hydroxyvitamin D (25-OHD) concentrations are largely determined by environmental factors, mainly through vitamin D intake and ultraviolet exposure. The sun is the primary source of vitamin D, which is synthesized endogenously in skin to produce cholecalciferol (vitamin D3), although a small proportion (<20%) of vitamin D comes through diet from a limited range of foods (in the form of ergocalciferol [vitamin D2] and vitamin D3).[2] The main marker of vitamin D status is the metabolite 25-OHD, which is synthesized in the liver.[3,4]

A relationship between type 1 diabetes mellitus and vitamin D deficiency has been reported.[5,6] The prevalence of vitamin D deficiency in patients with type 1 diabetes was 15% to 90.6%.[7-9] There is evidence that vitamin D is important in the prevention of islet cell death and might be useful in improving the survival of islet cell grafts, and it improves the production of insulin. Low vitamin D levels were shown to have a negative effect on beta-cell function.[3,4] Regular doses of vitamin D early in life have been shown to reduce the risk of developing type 1 diabetes.[5] Vitamin D treatment has also been shown to improve glycemic control and insulin sensitivity in people with type 1 and type 2 diabetes and in normal individuals. Increasing vitamin D levels from 25 to 75 nmol/L results in a 60% improvement in insulin sensitivity.[3,4,10-12] These effects have been mainly attributed to the immunomodulatory actions of vitamin D.[5]

We conducted a prospective, nonblinded and nonrandomized controlled trial to test the hypothesis that vitamin D supplementation would improve glycemic control in patients with type 1 diabetes who are vitamin D deficient.

PATIENTS AND METHODS

Patients aged more than 12 years who had type 1 diabetes mellitus were eligible.[1] Exclusion criteria were a history of liver disease, abnormal renal function, current use of vitamin D or calcium and pregnancy or breastfeeding. The doses of insulin were adjusted during the study. All patients were recruited from the King Fahad Armed Forces Hospital Diabetes Centre between January 2008 and June 2009. All patients gave their or their guardians' informed consent prior to inclusion. The study was approved by the ethical board of King Fahad Armed Forces Hospital. Eligible patients met the investigators 4 weeks before the start of the study for a complete history and physical examination and to have baseline laboratory assessments, including glycosylated hemoglobin, 25-OHD, calcium, phosphorus, magnesium and parathyroid hormone. The serum level of 25-OHD was measured by competitive protein-binding assay using appropriate kits (Immunodiagnostic, Bensheim, Germany). Glycosylated hemoglobin was measured using high-performance liquid chromatography.

Vitamin D deficiency was defined as a 25-OHD level of less than 50 nmol/L.[13] At entry into the study, patients having 25-OHD <50 nmol/L were assigned to receive 4000 IU of vitamin D and a total calcium intake of 1200 mg/d. All patients continued their current doses of insulin. The endpoints for glycemic control and vitamin D status were comparison of the glycosylated hemoglobin and 25-OHD levels at the beginning and at 12 weeks. Serum 25-OHD tertiles were defined as follow: first tertile (<35.4 nmol/L), second tertile (35.4-51 nmol/L) and the third tertile (>51 nmol/L). Glycosylated hemoglobin tertiles were defined as follows: first tertile (<7.8), second tertile (7.8-9.9%) and the third tertile (>9.9%).

Univariate analyses of baseline and follow-up demography and clinical laboratory endpoints were accomplished using the unpaired *t* test and paired *t* test where appropriate. The chi-square test was used for categorical data comparison. Pearson correlation coefficients between continuous variables were used as a measure of association. The data were analyzed by one-way analysis of variance (ANOVA), followed by post hoc LSD multiple comparison test to estimate the significance of difference between groups, and linear regression analysis was used as appropriate. All statistical analyses were performed using SPSS version 16.0. All P values were based on two-sided tests. The difference between groups was considered significant when *P* was <.05.

RESULTS

Eighty patients completed the study. Baseline characteristics (**Table 1**) were similar for each serum 25-OHD tertile (**Table 2**). There was also a significant difference in mean glycosylated hemoglobin level between the groups that achieved 25-hydroxyvitamin D levels of <35.4 nmol/L, 35.4-51 nmol/L and >51 nmol/L at 12 weeks (*P*=.02). There was a significant difference in glycosylated hemoglobin change from baseline for each tertile. The correlation of baseline and follow-up glycosylated hemoglobin levels in patients who achieved 25-hydroxyvitamin D levels of >51 nmol/L was -1.0 (95% C.I., -1.6, -0.5), *P*=.001, Pearson test. Mean (SD) glycosylated hemoglobin levels in patients who achieved 25-hydroxyvitamin D levels of >75 nmol/L as compared to those who achieved <75 nmol/L at 12 weeks were 7.3(1.5) vs. 9.1(2.4, respectively, *P*=.02. Patients were more likely to achieve lower glycosylated hemoglobin if they had higher 25-hydroxyvitamin D levels at 12 weeks (r= -0.4, *P*=.001, Pearson's test).

There was a significant difference in achieved 25-hydroxyvitamin D level between the groups that

Table 1. Baseline characteristics of study population.

Numbers	80
Male / Female	29 (36.3) / 51 (63.7)
Age (years)	21.0 (7.6)
Body mass index (kg /m²)	22.3 (4.0)
Diabetes duration (years)	7.1 (6.7)
Calcium (mmol/L)	2.3 (0.2)
Phosphorus (mmol/L)	1.3 (0.3)
Magnesium (mmol/L)	0.8 (0.1)
Parathyroid hormone (ng/L)	5.8 (6.1)
Glycosylated hemoglobin at base line (%)	9.4 (2.3)
25-hydroxyvitamin D level (nmol/L)	
<25	56 (70)
≥25	24 (30)

Data are mean (standard deviation) or number (percent).

Table 2. Baseline characteristics by 25-hydroxy vitamin D tertiles.

Parameters	25-hydroxyvitamin D level (nmol/L)			P
Values	<35.4	35.4-51	>51	
Numbers (%)	26 (32.5)	27 (33.8)	27 (33.8)	
Male/Female (%)	27.6/35.3	29.7/41.2	51.7/23.5	.03
Age (years)	19.4 (5.1)	20.5 (8.7)	23.0 (8.3)	.2
Body mass index (kg /m^2)	21.9 (4.7)	22.5 (4.0)	22.6 (3.4)	.6
Diabetes Duration (years)	8.4 (7.3)	7.1 (6.7)	6.5 (6.5)	.7
Calcium	2.4 (0.2)	2.3 (0.1)	2.3 (0.3)	.7
Phosphorus	1.3 (0.4)	1.3 (0.3)	1.3 (0.3)	1.0
Magnesium	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	.5
Parathyroid hormone	5.7 (4.4)	5.4 (2.7)	6.1 (9.2)	.9
Glycosylated hemoglobin				
Baseline	9.8 (2.2)	9.2 (2.4)	9.2 (2.4)	.6
Follow-up	10.0 (2.1)	9.0 (2.3)	8.2 (2.3)	.02
Change (95% CI)	-0.2 (-0.5, 0.8)	-0.2 (-1.1, 0.6)	-1.0 (-1.6, -0.5)	.04
25-hydroxyvitamin D level at follow-up (nmol/L)	24.5 (5.5)	44.6 (4.4)	75.1 (17.7)	<.0001

Data are mean (standard deviation) or number (percent).

Table 3. Glycosylated hemoglobin tertiles by 25-hydroxyvitamin D tertiles at 12 weeks.

		Glycosylated hemoglobin tertiles (%)								
		>9.9			7.8-9.9			<7.8		
		Female	Male	Total	Female	Male	Total	Female	Male	Total
25-hydroxy-vitamin D tertiles (nmol/L)	<35.4	50	50	50	33.3	37.5	34.6	16.7	12.5	15.4
	35.4-51	38.1	16.7	33.3	33.3	66.7	40.7	28.6	16.7	25.9
	>51	25	13.3	18.5	25	26.7	25.9	50	70	55.6

achieved glycosylated hemoglobin levels of <7.8, 7.8-9.9 and >9.9 at 12 weeks—61.9(23.4), 45.1(22.1) and 38.5(19.3), respectively, P=.001. Age and 25-hydroxyvitamin D levels at 12 weeks were significantly correlated in a linear regression with better glycosylated hemoglobin levels (−0.3, P=.006; and −0.4, P=.001, respectively). There were no significant differences in the mean and the tertiles of glycosylated hemoglobin between the groups that achieved 25-hydroxyvitamin D levels of <35.4 nmol/L, 35.4-51 nmol/L and >51 nmol/L at 12 weeks in both genders (**Tables 3 and 4**).

DISCUSSION

Diabetes mellitus has been recognized as a main independent risk factor for cardiovascular diseases.[14] Clinical studies indicate that most diabetic patients die due to cardiovascular diseases, with atherosclerosis accounting for about 8% to 10% of all diabetic deaths.[15] Diabetes mellitus is a complex, progressive disease, accompanied by multiple complications. Hyperglycemia has been accepted as being essential for the development of diabetic complications. The Diabetes Control and Complication Trial (DCCT) established that prolonged exposure to hyperglycemia is considered the primary factor associated with the development of diabetic macrovascular complications in type 1 diabetic patients.[16] The DCCT showed that improvement of glycemic control, as measured by reduction in glycosylated hemoglobin levels, significantly reduced the risk of development and/or

Table 4. Tertiles at 12 weeks by gender.

25-hydroxyvitamin D tertiles (nmol/L)	Glycosylated hemoglobin	Female	Male	*P*
<35.4	Baseline	9.8 (2.4)	9.8 (1.9)	.9
	Follow-up	10.1 (2.4)	9.7 (1.4)	.96
	Mean change	0.4	- 0.1	.5
35.4-51	Baseline	9.3 (2.6)	9.1 (1.7)	.9
	Follow-up	9.1 (2.5)	8.8 (1.6)	.8
	Mean change	- 0.2	- 0.3	.9
>51	Baseline	9.8 (2.2)	8.8 (2.5)	.3
	Follow-up	8.2 (2.7)	8.2 (2.1)	.9
	Mean change	-1.6	- 0.6	.05

Values are mean (standard deviation)

progression of all diabetic complications and also reduced the mortality and morbidity due to cardiovascular diseases in type 1 diabetes mellitus patients.

Insulin resistance plays a larger role in the type 1 diabetes disease process than is commonly recognized. Subsets of people with mild manifestations of the type 1 autoimmune disease process could benefit from treatments aimed at improving the insulin-resistant state.[17] There is evidence that vitamin D is important in the prevention of islet cell death.[18] Reports have shown the association of hypovitaminosis D with insulin resistance and beta-cell dysfunction,[3,19,20] and vitamin D is required to improve the production of insulin.[3,4] There are few studies that have examined the effect of supplementation with a variety of formulations of vitamin D on type 2 diabetes mellitus parameters. Among 18 young healthy men, supplementation with 1,25-(OH)2D3 for 7 days did not change fasting glycemia or insulin sensitivity.[21] In another small study of 14 patients with type 2 diabetes mellitus, 1-OHD3 administration daily for 3 weeks enhanced insulin secretion, but had no effect on post-load glucose tolerance.[22] Ljunghall et al randomized 65 middle-aged men with impaired glucose tolerance or mild diabetes and sufficient vitamin D levels at baseline to 0.75 g/d of 1-OHD3 or placebo for 3 months and found no effect on fasting or stimulated glucose tolerance.[23] In a crossover trial, 20 patients with type 2 diabetes mellitus and vitamin D deficiency were treated for 4 days with 1,25-OHD, and no change was seen in fasting or stimulated glucose, insulin or C-peptide concentrations.[24] Pittas et al have shown that insulin sensitivity is improved by as much as 60% when levels of vitamin D are increased from 25 to 75 nmol/L.[25] In a post hoc analysis of a 2-year trial, supplementation with vitamin D3 or 1-OHD3 had no effect on fasting glycemia in postmenopausal nondiabetic women.[26] One study reported that glycemic control became worse in three Asian patients following vitamin D supplementation;[27] however, these patients received vitamin D2 and not vitamin D3. Vitamin D2 has several unknown metabolites with unknown effects, and certain vitamin D receptor genotypes are big determinants of insulin secretory capacity in various ethnic groups.[28,29] Luo et al showed that among 109 patients aged over 50 years with type 2 diabetes who received cholecalciferol 2000 IU daily for 3 months, glycosylated hemoglobin concentrations and insulin use did not change significantly.[30]

The patients in our study were repleted with vitamin D3, and using up to 4000 IU of vitamin D3 to reverse states of deficiency was found to be safe.[31] There are no studies that have examined the effect of supplementation with a variety of formulations of vitamin D on glycemic control in type 1 diabetes mellitus. We are the first to have undertaken a study that has shown that the addition of vitamin D3 to insulin therapy produces a significant improvement in glycemic control. This effect on glycemic control was sustained over a period of 12 weeks; we do not know if this effect would be sustained further.

The study had some limitations. Increased hypoglycemia is a well-known complication of improved glucose control, and an important goal of therapy is to minimize this risk. Hypoglycemic episodes were not obtained by our patients. The study was neither blinded nor randomized, and the incremental doses of insulin were not analyzed.

In conclusion, diabetes is one of the fastest-growing chronic diseases worldwide. Vitamin D deficiency is common, and repletion might improve glycemic control in type 1 diabetes. Vitamin D3 is inexpensive and readily available. Well-designed clinical studies are required to ascertain if improving 25-OHD levels from deficiency to sufficiency improves glycemic control in patients with type 1 diabetes.

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original article

Effect of classic uvulopalatopharyngoplasty and laser-assisted uvulopalatopharyngoplasty on voice acoustics and speech nasalance

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BACKGROUND AND OBJECTIVE: Uvulopalatopharyngoplasty (UPPP) is a commonly used surgical technique for oropharyngeal reconstruction in patients with obstructive sleep apnea (OSA). This procedure can be done either through the classic or the laser-assisted uvulopalatopharyngoplasty (LAUP) technique. The purpose of this study was to evaluate the effect of classic UPPP and LAUP on acoustics of voice and speech nasalance, and to compare the effect of each operation on these two domains.

PATIENTS AND METHODS: The study included 27 patients with a mean age of 46 years. All patients were diagnosed with OSA based on polysomnographic examination. Patients were divided into two groups according to the type of surgical procedure. Fifteen patients underwent classic UPPP, whereas 12 patients were subjected to LAUP. A full assessment was done for all patients preoperatively and postoperatively, including auditory perceptual assessment (APA) of voice and speech, objective assessment using acoustic voice analysis and nasometry.

RESULTS: Auditory perceptual assessment of speech and voice, acoustic analysis of voice and nasometric analysis of speech did not show statistically significant differences between the preoperative and postoperative evaluations in either group ($P>.05$).

CONCLUSION: The results of this study demonstrated that in patients with OSA, the surgical technique, whether classic UPPP or LAUP, does not have significant effects on the patients' voice quality or their speech outcomes.

In patients with obstructive sleep apnea (OSA), uvulopalatopharyngoplasty (UPPP) is the most commonly used surgical procedure for enlarging the potential airspace in the oropharynx. The operation was first introduced by Fujita et al[1] as a new technique to correct anatomic abnormalities in OSA patients. Laser-assisted uvulopalatopharyngoplasty (LAUP), later developed by Kamami,[2] was aimed at progressively enlarging the oropharyngeal airspace by successive vaporizations of the uvula, soft palate, posterior tonsillar pillars and any redundant posterior pharyngeal mucosa. Postoperative pain, fatigue, hemorrhage, velopharyngeal insufficiency and nasopharyngeal stenosis were reported after both operations.

The phonatory characteristics after UPPP have been examined by different authors using objective (acoustical analysis) and subjective (perceptual evaluation) assessment techniques, but these studies mainly concentrated on the fundamental frequency, with contrasting results, and most of them investigated the effects of classical UPPP techniques.[3] Whereas a number of authors reported significant voice changes after UPPP, other authors found no differences in voice and speech characteristics.[4] There are only a few studies that have tested changes in voice and speech quality in patients with OSA after different palatal surgical techniques; therefore, uncertainty exists about speech and voice status in patients following UPPP and LAUP surgery. The aim of this study was to evaluate the effect of classic UPPP and LAUP on acoustics of voice and speech nasalance, and to compare the effect of each operation on these two domains to establish new concepts of management and a rationale for a therapeutic approach.

PATIENTS AND METHODS

The study was carried out at Ain Shams University Hospital, Cairo, Egypt. Twenty-seven adult patients (20 males and 7 females) with OSA who presented to the otolaryngology clinic between May 2007 and August 2008 were included in this study after having consented to participate. Their ages ranged from 18 to 57 years. Patients were selected when OSA was suspected based on history, clinical examination and polysomnography. According to the respiratory distress index (RDI), 7 patients had simple snoring without OSA (RDI, <5 events/hour), 5 patients had mild OSA (RDI, 5-25 events/hour), 7 patients had moderate OSA (RDI, 25-50 events/hour) and 8 patients had severe OSA (RDI, >50 events/hour). Patients subjected to previous operations for snoring and/or sleep apnea were excluded. In all subjects, OSA or severe snoring was established by polysomnographic examination. Each subject was assessed by an otorhinolaryngologist performing a complete ear, nose and throat examination to exclude voice disorders and nasal and ear diseases. For the nasopharyngeal and laryngeal examination, a fiberoptic nasopharyngolaryngoscope was used. The patients were randomly divided into two groups: 15 patients underwent classic UPPP, whereas 12 patients were subjected to LAUP. UPPP is a procedure designed to enlarge the potential airspace in the oropharynx. The uvula and part of the free edge of the soft palate were removed under general anesthesia. The procedure of LAUP operation was performed in the office using local anesthesia and a carbon dioxide (CO_2) laser with special hand pieces to reduce and reshape the uvula and soft palate. Full-thickness vertical incisions were made on the edge of the soft palate approximately 1 to 1.5 cm in length on both sides of the uvula using CO_2 laser at a power of 16 W in continuous mode. The resultant elongated uvula was then lasered off. The procedure progressively enlarges the oropharyngeal airspace by successive vaporizations of the uvula, soft palate, posterior tonsillar pillars and any redundant posterior pharyngeal mucosa. The following investigations were done for all the patients preoperatively and 6 months postoperatively.

Auditory perceptual assessment (APA)

Standardized speech samples were recorded using a high-fidelity tape recorder in a sound-treated room. The speech samples included a standardized passage, counting from 1 to 10 and sustaining the vowels /a/, /i/, and /u/. The recorded speech samples were perceptually judged for voice changes and nasality by three experienced phoniatricians. The degree of hypernasality was evaluated using a five-point scale (0=normal and 4=severe). Auditory perceptual assessment (APA) of voice was done according to the GRBAS (grade, roughness, breathiness, asthenia, strain) scale. Also, the parameters for overall grade of dysphonia, roughness and breathiness were rated on a five-point scale (0=normal and 4=severe).

Acoustic analysis of voice

A computerized speech lab (CSL model 4300 from Kay Elemetrics Corp., Lincoln Park, NJ, USA) was used for acoustic analysis of voice. The patient was seated in front of the microphone, and the mouth-microphone distance was kept constant at about 10 cm. The patient was asked to produce the sustained vowel /a/ in a flat tone at a comfortable pitch and at constant amplitude. Average pitch, jitter, shimmer and harmonic-to-noise ratio were measured for the two groups. Formant frequencies were done for the vowels /a/, /i/ and /u/. The first 2 formants (F1 and F2) were tested for these vowels. Acoustic analysis of voice was done preoperatively and 6 months postoperatively for all patients in both groups.

Acoustic analysis of speech (nasometric evaluation)

Using a nasometer (Model 6200 from Kay Elemetrics Corp., Lincoln Park, NJ, USA), the patient was instructed to produce a standardized oral sentence and a nasal sentence adapted to the Arabic language. Nasometric analysis of the patient's speech was conducted to monitor possible postoperative nasality changes after operative procedures for each group.

The results were statistically evaluated by comparing the mean values of each preoperative and postoperative parameter for each operation, using the paired t test. The significance level was set to .05 throughout. The Statistical Package for Social Sciences, version 11 (SPSS Inc., Chicago, IL), was used for all statistical analyses.

RESULTS

Twenty-seven patients participated in the study. Two patients were excluded, one because of an articulation disorder, and the other because of a common cold and nasal congestion on the day of testing. The selected group of subjects included 25 patients who completed the whole protocol of assessment preoperatively and postoperatively. The age of the study group ranged from 31 to 67 years (mean age, 46.6 years). There were 20 males and 7 females.

Table 1 shows the APA results of the patients in both groups preoperatively and postoperatively. Differences in voice quality were not significant between

Table 1. Comparison of preoperative and postoperative auditory perceptual assessments of voice.

Parameter	UPPP		*P*	LAUP		*P*
	Preop	Postop		Preop	Postop	
Overall grade	0.3 (3)	0.2 (3)	>.05	0.5 (0.4)	0.3 (0.3)	>.05
Roughness	1.2 (0.1)	1.5 (0.2)	>.05	0.91 (0.12)	1.2 (0.14)	>.05
Breathiness	0.4 (0.25)	0.51 (0.2)	>.05	0.61 (0.1)	0.53 (0.24)	>.05
Strain	0.8 (3)	0.6 (2)	>.05	1.59 (0.5)	1.3 (0.3)	>.05

Values are mean (standard deviation).

Table 2. Comparison of preoperative and postoperative values of acoustic parameters of voice.

Parameter	UPPP		*P*	LAUP		*P*
	Preop	Postop		Preop	Postop	
Average pitch (Hz)	167 (29)	165 (32)	>.05	170 (38)	168 (40)	>.05
Jitter (%)	0.21 (0.1)	0.19 (0.1)	>.05	0.13 (0.12)	0.13 (0.14)	>.05
Shimmer (%)	0.4 (0.25)	0.51 (0.2)	>.05	0.61 (0.1)	0.53 (0.25)	>.05
H/N (dB)	12.2 (3)	12.18 (3)	>.05	13.9 (3.5)	12.9 (4)	>.05

Values are mean (standard deviation).

Table 3. Comparison of preoperative and postoperative nasalance scores (%).

Parameter	UPPP		*P*	LAUP		*P*
	Preop	Postop		Preop	Postop	
Oral sentence	9.49 (1.19)	9.58 (1.03)	>.05	9.3 (1.5)	10.5 (3.1)	>.05
Nasal sentence	44.8 (4.3)	44.7 (3.5)	>.05	42.6 (4.48)	45.3 (9.0)	>.05

Values are mean (standard deviation).

Table 4. Comparison of preoperative and postoperative values of first formant (F1) and second formant (F2) of vowels /i/, /u/ and /a/ in patients subjected to UPPP.

Vowels	F1		*P*	F2		*P*
	Preop	Postop		Preop	Postop	
/i/	299 (22)	279 (77)	>.05	1808 (83)	1408 (78)	>.05
/u/	335 (30)	336 (27)	>.05	767 (46)	766 (43)	>.05
/a/	522 (143)	520 (140)	>.05	971 (39)	965 (48)	>.05

Values are mean (standard deviation).

Table 5. Comparison of preoperative and postoperative values of first formant (F1) and second formant (F2) of vowels /i/, /u/ and /a/ in patients subjected to LAUP.

Vowels	F1		*P*	F2		*P*
	Preop	Postop		Preop	Postop	
/i/	310 (21)	314 (20)	>.05	1841 (69)	1850 (58)	>.05
/u/	348 (20)	346 (21)	>.05	788 (18)	785 (17)	>.05
/a/	558 (30)	556 (32)	>.05	963 (34)	960 (33)	>.05

Values are mean (standard deviation).

the preoperative and postoperative evaluations for patients in each group (UPPP and LAUP). There was no statistically significant difference in either group between preoperative and postoperative assessments of acoustic quality. The *t* test showed no statistically significant change in average pitch, jitter, shimmer and harmonic-to-noise ratio before and after UPPP and LAUP for the sound /a/ (**Table 2**). Nasometric assessment did not show any statistically significant difference between the preoperative and postoperative nasalance scores in patients subjected to UPPP or those who underwent LAUP (**Table 3**). The formants F1 and F2 in each study group did not demonstrate any statistically significant changes between preoperative and postoperative assessments. The paired *t* test showed insignificant preoperative and postoperative differences in F1 and F2 values for /i/, /u/ and /a/ vowels in patients subjected to either UPPP (**Table 4**) or LAUP (**Table 5**).

DISCUSSION

Patients and physicians are always worried about the effect of surgical modalities used for treatment of OSA on voice and speech. In a previous study performed by Mora et al,[5] they found improvement in acoustic parameters and nasalance scores in all patients who underwent UPPP with the harmonic scalpel, 6 months after the operation, and they reported improvement in patients' voice and speech after the operation. However, in our study, APA of the voice revealed no statistically significant voice changes postoperatively compared to the preoperative assessment. This was demonstrated in all patients who underwent either UPPP or LAUP. Our results also revealed that there were no statistically significant differences in acoustic voice parameters (pitch, jitter, shimmer and harmonic-to-noise ratio) among all patients before and after either UPPP or LAUP. The possible explanation for these findings is that acoustic voice parameters, including fundamental frequency and perturbation in frequency and loudness, reflect mainly the status of vocal folds' vibration; however, the surgical technique, whether UPPP or LAUP, does not have a direct effect on the vocal folds or the larynx. This concurs with the findings by Van Lierde et al[6] in their study of the voice and speech changes after UPPP. They found insignificant changes in the tested groups before and after the operation.

No statistically significant differences were found between preoperative and postoperative assessments in patients who underwent either UPPP or LAUP in nasalance of oral and nasal sentence. This means that the operative techniques did not affect the patients' speech quality in terms of the nasal resonance. This concurs with the study done by Rihkanen and Soini,[7] who mentioned that UPPP should not have a significant effect on speech characteristics as long as excessive nasality is not produced. On the other hand, Keilmann et al[8] found that most of their patients had hypernasality and nasal escape of air 1 week after UPPP. Three to four months later, only one patient had hypernasality. Almost 1 year after surgery, velopharyngeal incompetence was no longer detected. This could be explained by the presence of functional hypernasality in the postoperative period due to the pain experienced postoperatively in the velum. However, later on the velopharyngeal valve worked competently in almost all patients.

The formants are features of the resonating cavities lying above the vocal folds. The formant is determined by the length and shape of the vocal tract, as these cavities are reshaped by changes in the positions of the tongue, jaw and lips. The first and second formants (F1 and F2) have frequencies for different vowels that generally reflect differences in tongue articulation. F1 frequency is related to the articulatory feature of tongue height. The vowels with a back tongue position have a low F2 frequency, whereas vowels with a front tongue position have a high F2 frequency. Thus F2 is generally related to the feature of tongue advancement (position of the tongue body on a back-front dimension).[9]

In the present study, on studying the preoperative and postoperative analysis of F1 and F2 values of different vowels (/i/, /u/ and /a/), we found no statistically significant differences between patients who underwent either UPPP or LAUP. This may be explained by the fact that F1 and F2 may be affected by the tongue shape and position and are not related to the shape of the soft palate.

The sample size of this study was relatively small, which is a potential limitation, but related studies have included almost similar number of patients.[5,6,10] Those studies revealed results comparable to those of our study on the effect of UPPP on voice acoustics and speech nasalance.

In conclusion, classic UPPP and LAUP are two different surgical techniques used for oropharyngeal reconstruction in patients with OSA. The results of this study revealed that the surgical technique, whether classic UPPP or LAUP, does not have any significant effects, either on the patients' voice quality or on the nasal resonance of their speech.

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Hypoglycemia and safe driving

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The lack of awareness of the effects of hypoglycemia on safe driving is a real issue for diabetic patients and a challenge for health care providers. Taking the form of questions and answers, this review addresses the issue of road traffic accidents and drivers with type 1 diabetes mellitus. While there is little evidence showing higher accident rates among diabetic drivers, there is research indicating that hypoglycemia compromises driving performance, resulting in slower response times and reduced cognitive function. Unawareness of an early fall in plasma glucose is another important issue that affects some diabetic drivers. The driver with type 1 diabetes is obliged to check their blood glucose before driving. The physician's duty is to familiarize the patient with the risk of hypoglycemia. If hypoglycemic unawareness is present, the physician should advise the patient to stop driving until the condition is reversed. The doctor should consider informing authorities if he concludes there is a risk and the driver cannot be persuaded to stop driving.

In this review, the subject of hypoglycemia and safe driving is presented in the form of a series of questions. These are questions that might be asked of a diabetologist called as an expert witness in a trial involving a driver with type 1 diabetes who has caused an accident by dangerous driving.

Q 1. What is the effect of hypoglycemia on mental status?

Investigations into the effect of glucose on performance have been conducted in humans. Some studies have explored the effect of glucose on learning, memory and mood in school children; on attention, memory and decision making in college students; and on memory in adults. Benton, Parker and Donohoe[1] provided a useful overall review of the effect of blood glucose on cognitive functioning. They discussed the widely accepted view that hypoglycemia causes physical and psychological symptoms associated with disruption of cognitive function.[1] Hypoglycemia has been found to induce adrenergic symptoms such as nervousness and tremor, as well as tiredness, confusion and retarded mental function.[2] The first signs and symptoms of low blood sugar can begin to occur when serum glucose drops under 70 mg/dL, although this varies from person to person. Comi stated that complete cognitive recovery may lag for 30 to 45 minutes behind restoration of normal blood glucose levels.[3] Safe driving requires constant integration of mental function (visual and auditory processing, or motor skills; reasoning, logic or problem solving).

Certain visual functions are affected at low blood glucose levels, e.g., detection of visual change and movement and the reaction time to visual stimuli.[4] Performance of visual tasks requiring more involved processing was found to be adversely affected at low blood glucose levels. Also, decision making ability based on auditory processes was likewise impaired at low blood glucose levels.[5] Aspects of attention are affected at low blood glucose levels: speed of performance on attention-requiring tasks was reduced when blood glucose levels were low, and subjects then became slower in their reaction response.[6]

Reaction time in the performance of vigilance tasks, detection of auditory or visual tones and tracking tasks was significantly lower at low blood glucose levels.[7] Planning performance was slower, and mental flexibility and tracking were impaired at low blood glucose levels.[8] Low blood glucose levels significantly impaired complex task performance, assessed by driving simulation.[9] Steering control, speed control and braking were negatively affected at low blood glucose levels. Accuracy of performance was preserved at the expense of speed in these tasks, with very slow driving being demonstrated. Interestingly, patients with hypoglycemia are not always aware of their impairments.[10]

Q2. For a driver with type 1 diabetes, are there risks associated with driving?

No randomized controlled studies (which provide the strongest evidence) exist. Sophisticated driving simulator studies with demanding scenarios showed that driving in itself is a significant stressor that is associated with greater autonomic symptoms, higher epinephrine levels and higher glucose need.[11] Cox, Gonder-Frederick, Kovatchev, and Clarke found that actual driving was associated with a higher dextrose infusion rate (P=.02), more autonomic symptoms (P<.05), increased heart rate (P<.001), a trend toward greater epinephrine release (P=.09) and more frequent hypoglycemic self-treated (P<.001).[11]

Driving puts individuals with type 1 diabetes at increased risk of hypoglycemia.[11] Simulator performance studies showed that during hypoglycemia (even if mild with blood glucose between 4.0 and 3.4 mmol/L), persons with type 1 diabetes engage in some form of less safe driving (driving across the midline, speeding and/or inappropriate braking).[2] The exact blood glucose range at which driving is impaired and the exact driving parameters disrupted were found to be idiosyncratic.[10]

Depression of CNS activity (evidenced by increased theta wave activity in EEG) occurs at moderate hypoglycemia (3.3-2.8 mmol/L).[10] Moreover, such studies revealed that 23% to 43% of type 1 diabetes patients with awareness of impaired hypoglycemia (based on epinephrine level and symptoms score assessed by various neuropsychological tests) failed to decide not to drive during hypoglycemia, increasing the chance of dangerous driving.[12,13]

Functions that are mainly affected by hypoglycemia include rapid decision making, sustained attention, analysis of complex visual stimuli and hand-eye coordination.[14] In patients with type 1 diabetes and repeated hypoglycemia, there is marked deterioration of performance in tests of short-term, delayed and working memory for both verbal and nonverbal material.[15]

Q3. Is there an increased risk of accidents among diabetic drivers?

This has been controversial, but a recent retrospective study showed that significantly more drivers with type 1 diabetes made at least one auto crash over the preceding 2-year period as compared to drivers with type 2 diabetes or nondiabetic spouses (19% vs. 12% vs. 8%, respectively; P<.001).[7] Also, significantly more of those drivers with type 1 diabetes reported episodes of mild symptomatic hypoglycemia, hypoglycemic stupor and required assistance while driving due to development of hypoglycemia than the type 2 diabetic or nondiabetic drivers.[16] Type 1 diabetes drivers also reported twice as many crashes as their nondiabetic spouses.[16] Type 1 diabetes drivers who did not self-treat had almost three times the crash rate. The three main factors that significantly contributed to an increased probability of crashes were experiencing more episodes of hypoglycemic stupor while driving, less frequent self-monitored blood glucose before driving and taking insulin by subcutaneous injections rather than an insulin pump.[16]

Q4. Can you ascertain that a type 1 diabetic was in an accident suffering from a hypoglycemic attack?

It is difficult to ascertain, but clues to significantly increased possibility of a hypoglycemic stupor while driving in type 1 diabetes are accepting a lower blood glucose as a threshold for deciding not to drive and suffering more frequent episodes of mild symptomatic hypoglycemia while driving.[16] However, a diabetic with tight glycemic control (evidenced by glycated hemoglobin) is at more risk of hypoglycemia. The presence of hypoglycemia unawareness indicates the occurrence of previous severe hypoglycemia and raises the possibility that our patient suffered an attack while driving. The blood glucose in relation to driving and the time factor for development of events (in view of the recommendations) is necessary to judge further.

Q5. What must have happened before getting into a state of hypoglycemic unawareness?

Hypoglycemia unawareness is when a subject is unaware of an early fall in plasma glucose concentration and does not recognize the warning neuroglycopenic symptoms and fails to compensate by eating to prevent progression to severe hypoglycemia. The syndrome is the result of deficient sympathetic neural and adreno-medullary responses to falling glucose levels. Hypoglycemia unawareness can occur in a longstanding diabetic patient who has a defect in secretion of counter-regulatory hormones. It develops if there are frequent bouts of hypoglycemia (level below 3.0 mmol/L).[17]

Q6. Can a person know that he himself is prone to hypoglycemic unawareness?

By definition, a patient with hypoglycemia unawareness cannot recognize this state; full assessment can be completed by interviewing persons close to the patient in everyday life. Health education, self-monitoring of blood glucose can help, especially after exercise and during sleep.[17]

Q7. Can people go straight into hypoglycemic unawareness without previously experiencing a symptomatic episode of hypoglycemia? How many such previous symptomatic episodes may occur before the development of hypoglycemia unawareness?

Hypoglycemia unawareness develops only after recurrent bouts of hypoglycemic attacks.[18] Luckily this can be reversed by scrupulous avoidance of hypoglycemia in daily living. At least a 3-week period of meticulous avoidance of hypoglycemia should be attempted with the goal of encouraging a return to awareness of hypoglycemia.[19]

Q8. Can diabetes cause changes in mood and behavior? How is this related to driving?

Hypoglycemia promotes mood changes, including increased irritability and anger.[19,20] Long-term consequences can include a pattern of fear of hypoglycemia with a negative impact on the patient's health-related quality of life.[6] Patients with type 2 diabetes may experience feelings of depression and anxiety, which may affect their driving performance.[21]

Q9. Does hypoglycemia disrupt performance of different cognitive functions or impair nonverbal intelligence? And how does this affect driving?

Acute hypoglycemia causes progressive, reversible deterioration in cognitive function that becomes detectable with glucose below 3 mmol/L.[20,22,23] Cognitive function does not return to normal until 40 to 90 minutes after normoglycemia has been restored.[14,24]

Q10. Are there any specific obligations or recommendations that a driver needs to fulfill to ensure safe driving?

Yes, and these should be discussed by his physician and other members of the diabetic team treating the patient. Our type 1 diabetes driver should measure his blood glucose before driving and not drive if it is below 5 mmol/L[16,25] and should frequently recheck his blood glucose during long-journey driving.[16,25] (A 2-hour interval has been suggested by some.[7]) He should discontinue driving and consume a rapid-acting carbohydrate if hypoglycemic.[16,25] He should not continue driving until 30 to 45 minutes after restoration of blood glucose, as studies have shown that cognitive function may not recover until then.[16] A study where participants did blood glucose awareness training showed a two-thirds reduction in crashes and motor vehicle violations at long-term follow-up.[25] Type 1 diabetic drivers should be urged to wear potentially life-saving 'diabetes alert' identification, which will enable the rescue team to save his life and identify his blood sugar level at the moment of the accident.

Q11. What is the doctor's duty when treating a type 1 diabetes driver? And what precautions are advised?

Physicians have a duty to familiarize drivers with the risk of hypoglycemia and recognize its signs and symptoms.[26] Drivers should perform self monitoring, especially when long journeys are planned. This should be correlated with meals. Drivers should be strongly advised against risk-taking behavior. If the history is suggestive of hypoglycemic unawareness, the driver should be advised to stop driving until the condition is reversed, by scrupulously avoiding further hypoglycemia for at least 2 weeks.[27,28]

Q12 .What are the legal responsibilities of diabetic drivers and the doctor in relation to driver and vehicle licensing agency regulations?

The diabetic driver on insulin has a duty to inform the authorities about his diabetes. If he is on diet and/or oral hypoglycemic medication and is free of complications such as hypoglycemic unawareness, there is no need to inform the authorities. Generally, insulin-treated people are not allowed to drive heavy goods vehicles or certain passenger vehicles like buses. Approvals may be sought for exceptional cases.[29]

Although the onus is on the driver to inform, the doctor may on grounds of public interest inform the authorities if he concludes that there is a risk and if the driver cannot be persuaded to notify the authorities and to stop driving. This intention must be communicated to the patient. The driver and vehicle licensing agency also recommends discussing with the next of kin, but the patient is unlikely to agree. A second opinion may be sought; but until this is resolved, the patient should also refrain from driving.[29] The onus seems to rest largely on the individual patient, after due advice has been availed of and discussion has taken place. The legal position of the physician who fails to notify the authorities about a recalcitrant driver in the event of an accident is still untested.[29]

Conclusion

There is insufficient evidence to prove that diabetic drivers have higher accident rates.[30,31] However, some research supports the idea that hypoglycemia significantly compromises driving performance, resulting in longer response times and lower scores in cognitive tests,[10] which may lead to traffic violations and accidents.

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brief report

Predictive value of the cow's milk skin prick test in infantile colic

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BACKGROUND AND OBJECTIVES: Infantile colic is a common problem among young infants. Cow's milk allergy has been suggested as one of the causes. We aimed to investigate the value of the cow's milk skin test for the diagnosis of cow's milk allergy in exclusively breast-fed infants with infantile colic.

METHODS: Exclusively breast-fed infants with infantile colic were enrolled in this study. On the first visit, the average hours of crying of the infant in a 24-h period were recorded and the cow's milk skin test was performed. If the infant had a positive skin test, elimination of cow's milk from the mothers' diet was advised. Infants with negative skin tests were divided into case and control groups. Cow's milk was eliminated from the diet of mothers in the case group. After 2 weeks, the number of hours of crying were recorded again. The reduction in the crying hours was compared between the two groups using the chi-square test.

RESULTS: Skin tests were positive in 3 of 114 cases (2.6%) of infantile colic. All three cases recovered completely following elimination of cow's milk from the mother's diet. Among the 111 patients with negative skin tests, 77 patients completed the study: 35 in the case group and 42 in the control group. The reduction in crying hours in infants in the case group was not significantly different from that in the control group.

CONCLUSION: Elimination of cow's milk from the mothers' diet is not beneficial for infants with a negative skin test. Infants with a positive skin test may benefit from this management.

Infantile colic affects up to 28% of infants in the first few months of life.[1,2] Frequent episodes of crying in these infants may cause significant disruption of family interactions.[3-6] Food allergy is a common problem in infants and small children. The classical allergist's tool for diagnosing allergies is skin prick testing (SPT), and this can also be applied for diagnosis of food allergy.[7] Despite the availability of various skin and laboratory tests, the current clinically valid method to demonstrate significant food allergy is elimination and challenge with the suspected food.[8] Cow's milk protein is the most common food allergen in infants under 1 year of age. Due to an increasing number of infants in need of diagnostic workup, it has become acceptable to diagnose cow's milk allergy in infants on the basis of a positive SPT and cessation of symptoms on an elimination diet.[9]

The role of cow's milk allergy in infantile colic is controversial.[10] Some studies have reported a reduction in persistent crying after elimination of cow's milk and other food proteins from the mother's diet,[11,12] but other studies have reported that maternal diet does not seem to be related to infantile colic in breast-fed infants.[13,14] In the present study, we aimed to investigate the value of SPT in the diagnosis of cow's milk allergy in patients with infantile colic.

METHODS

This single-blind randomized clinical trial was done on exclusively breast-fed infants with history of infantile colic. All suspected cases of infantile colic between the ages of 3 weeks and 3 months were seen by a pediatrician, who diagnosed infantile colic on the basis of a complete history and thorough physical examination. Other probable etiologies were ruled out. Diagnosis of infantile colic was made on the basis of Wessel criteria, i.e., severe crying in infants between 3 weeks and 3 months old, occurring at least 3 days per week and lasting 3 hours per day.[15]

For each infant, the parent(s) were asked about the average number of hours of crying in 24 hours and this was recorded. After explaining the nature of the study to the parents and obtaining written informed consent, SPT was performed for all the infants. SPT was done on the volar aspect of the forearm by an expert nurse, under the close supervision of a pediatric allergist, with cow's milk extract (Allergopharma, Germany), fresh cow's milk, histamine dihydrochloride (as a positive control), and glycerol (as a negative control). All necessary precautions for management of any possible adverse reaction were taken. Reactions were read after 15 minutes, and a response was considered positive if the mean diameter of the wheal was at least 3 mm more than the negative control.

Infants with a positive SPT were excluded. The rest were divided randomly into case and control groups. For infants in the control group, no change in the mother's diet was suggested and the parents were instructed to bring their babies for follow-up 2 weeks later. Mothers of infants in the case group were asked to avoid cow and goat milk as well as dairy products for 2 weeks. These mothers were prescribed calcium supplements and were instructed to take a calcium-rich diet. The infants in the case group were also seen again at follow-up 2 weeks later.

On the second visit, the same parents were asked about the average number of hours of crying per day, and the infants were reexamined by a pediatrician who did not know the group allocation of the infants. Those infants whose crying hours were decreased by 1–2 hours per day were labeled as partial responders, and decrease in crying hours by more than 2 hours per day was considered a complete response. No difference in number of crying hours or a decrease of less than 1 hour per day was labeled as no response. Infants whose parents did not follow all of the instructions and those who were not brought for follow-up after the first visit were excluded from the study. The responses of the case and control groups were compared using the chi-square test. P=.05 indicated statistical significance.

RESULTS

Of 153 exclusively breast-fed infants with colic, the parents of 114 cases allowed us to do the SPT. Among these infants, three cases had a positive SPT (2.6%). For these three cases, elimination of cow's milk products from the maternal diet resulted in complete recovery. The 111 SPT-negative cases were randomly divided into case and control groups. Of these infants, 35 infants of the case group (mothers asked to avoid cow and goat milk and other dairy products) and 42 infants of the control group (regular diet) returned for follow-up after 2 weeks. The different stages of the study are detailed in **Figure 1**. The change in the number of crying hours in the case and control groups were compared using the chi-square test, but no statistically significant difference was seen between the two groups (**Table 1**).

DISCUSSION

In this study, the effect of cow's milk allergy was evaluated in infantile colic through two methods: elimination diet and SPT. SPT with cow's milk was performed for 114 cases with infantile colic and was positive in 2.6% of cases. Previous studies have demonstrated that SPT has a high specificity and low sensitivity for immediate-type food reactions in infants under the age of 1 year.[9] Indeed, a positive test in these infants suggests a relevant food allergy.[16] To the best of our knowledge, there are no previous studies of SPT in patients with infantile colic.

In this study the elimination of cow's milk and dairy products from the diet of mothers of exclusively breast-fed infants with negative SPT had no effect on the attacks of colic. The effect of a low-allergen diet on infan-

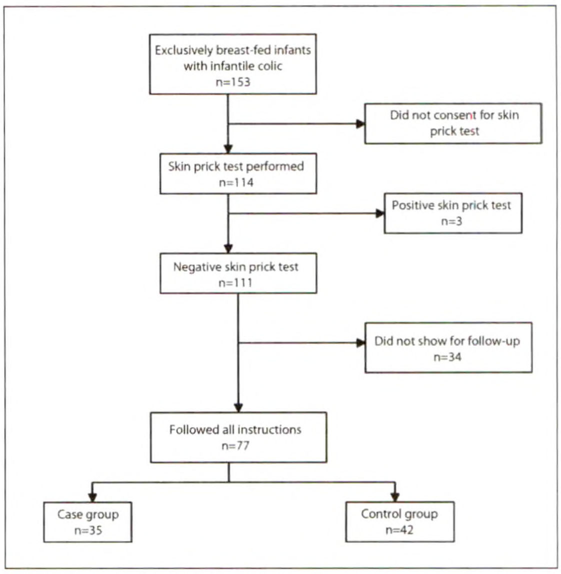
Exclusively breast-fed infants with infantile colic n=153
Did not consent for skin prick test
Skin prick test performed n=114
Positive skin prick test n=3
Negative skin prick test n=111
Did not show for follow-up n=34
Followed all instructions n=77
Case group n=35
Control group n=42

Figure 1. Stages of the study.

Table 1. Comparison of response rate between case and control groups.

Response	Case	Control
Complete response	14	11
Partial response	10	17
No response	11	14
Total	**35**	**42**

(Chi-square=1.19; P=.38)

tile colic has been studied in multiple studies and has shown different results. Hill et al studied the effect of a low-allergen diet on colic among breast-fed infants.[17] They instructed mothers to exclude multiple nutrients from their diets for a duration of 1 week and found that a low-allergen maternal diet is associated with a significant reduction in the time the infant spends in crying/fussing. It is clear that elimination diets have associated risks, particularly if sustained for long periods,[18] and elimination of multiple nutrients may not be a proper regimen for management in all cases.

Evans and coworkers[13] performed a similar study and concluded that maternal diet does not affect infantile colic in breast-fed infants. Taubman[19] compared parental counseling with elimination of cow's milk from the diet of infants and concluded that parental counseling is more effective than elimination of cow's milk for treatment of infantile colic. Garrison and Christakis[14] conducted a systematic review of the management of infantile colic and concluded that there is no conclusive evidence of the efficacy of a low-allergen diet in the management of infantile colic in exclusively breast-fed infants and that more studies are needed.We found that elimination of cow's milk from the mothers' diet has no effects on the symptoms of colic in infants with a negative SPT. Infants with positive SPT may benefit from elimination of cow's milk products from the maternal diet. More studies are needed to determine which patient will benefit from an elimination diet.

Our study had several limitations. There was a high dropout rate and the number of positive SPTs was low. Because of the nature of the intervention, the study could not be conducted as a double-blind trial. Furthermore, although the gold standard method for detection of food allergy is a double-blind placebo-controlled challenge test, because of the self-limiting nature of colic, the challenge test was not conducted in these infants.

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Infantile and early childhood masturbation: Sex hormones and clinical profile

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BACKGROUND AND OBJECTIVES: Few studies have explored the hormonal triggers for masturbation in infants and young children. Thus, we aimed to study the sex hormones and clinical profiles of masturbating infants and young children.

METHODS: This case-control study involved infants and young children who masturbate and were referred to three pediatric neurology clinics between September 2004 and 2006 (n=13), and a similar control group. All children underwent basic laboratory investigations prior to referral. Other tests included electroencephalography (n=8) and brain neuroimaging (n=9). We measured dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, free testosterone, estradiol, dehydroepiandrosterone, sex hormone-binding globulin (SHBG), and androstenedione in all participants.

RESULT: The median age at the first incident was 19.5 months (range, 4-36 months); the median masturbation frequency, 4 times/day; and the median duration of each event, 3.9 min. The subjects masturbated in both prone (n=10) and supine positions (n=3); two subjects used the knee-chest position. All subjects showed facial flushing; 6, friction between the thighs; 5, sweating; 9, sleeping after the event; and 12, disturbance on interruption. EEG was abnormal in one of eight subjects tested, and neuroimages were normal in all of nine subjects examined. The case and control groups had comparable levels of all sex hormones, except estradiol, which showed significantly lower levels in the case group (P=.02).

CONCLUSION: Masturbation in children seems to be associated with reduced estradiol levels, but not with other sex hormones. Further studies are needed to confirm our findings.

Childhood masturbation was reported by Still in 1909.[1] It is characterized by self-stimulation of the genitalia frequently associated with unusual posture and movement, sweating, flushing, tachypnea, and typically begins in infancy and early childhood.[2] Masturbation is a normal behavior in adolescence, occuring in 90% to 94% of males and 50% to 60% of females at some point in their lives; maturation of sex hormones predisposes to the activity.[3-5] Previous reports on infants and early childhood masturbation are sparse with no attempts to identify the role of sex hormones in such a situation, despite the evidence that sex hormones are known to predispose to adolescentmasturbation behavior.[5,6] In infants and young children, unusual postures and movements occur during masturbation and may be misdiagnosed as seizures, movement disorders, abdominal pain, colic, or other neurologic or medical problems.[7-10] Extensive unwarranted investigations may be performed.[10-12] To our knowledge, assessment of the levels of sex hormones as a possible predisposing factor has not been carried out before. The purpose of this study was to describe the clinical characteristics of masturbatory behaviors in 13 children referred to three different child neurology clinics in Jordan, to assess their sex hormones levels, and to compare these with that of a control group.

METHODS

This was a prospective study of all infants and young children referred to the participating pediatric neurology clinics between September 2004 and 2006, diagnosed as having gratification disorder. A data collection sheet was developed which included information on demographic characteristics, a detailed history on the features of the movements during masturbation, clinical examination, neurodevelopment assessment, as well as the levels of sex hormones (dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone [17OHP], free testosterone, estradiol, dehydroepiandrosterone (DHEA), sex hormone-binding globulin [SHBG], and androstenedione). All hormone levels were determined using commercially available kits as follows: DHEAS and 17OHP were measured by RIA, DHGA by immunoradiometric assay (Immunotech Marseille, France), free testosterone by RIA (Biosource Europe S.A., Belgium), estradiol by a microparticle enzyme immunoassay using an AXsym machine (Abbott Labs, IL, USA), SHBG by an enzyme immunoassay using a Cobas machine (Roche Diagnostics, Mannheim, Germany), and androstenedione by an enzyme immunoassay (DSL, Dallas, TX, USA). To assess the role of sex hormones in this condition, 13 age- and sex-matched controls were selected from children attending the same clinics for reasons other than masturbation. Blood samples were obtained from all controls and assessed for sex hormones using the same techniques as for the cases. The mean levels of the sex hormones were compared between cases and controls using the two-sample independent t test. Other tests performed included EEG (n=8), brain CT scan (n=8), and brain MRI (n=1). Five of the children were wrongly diagnosed as having epilepsy and were on a maintenance antiepileptic drug treatment prior to referral to our clinics. Basic blood tests included complete blood count, serum electrolytes, and liver and kidney profiles. Echocardiography was performed on all children before the referral. The ethics committees of the three institutes approved the study protocol. A verbal consent was obtained from the parents of all participants.

RESULTS

Thirteen infants and young children exhibiting masturbation were enrolled in this study. **Table 1** shows the characteristic features of events associated with masturbation. Antiepileptic drugs were given to five of the children, but did not have in any clinical benefit. A comparison of sex hormone levels between all cases and controls is shown in **Table 2. Table 3** shows the corresponding values in female cases. As shown in **Tables 2 and 3**, estradiol levels were found to be significantly lower in cases as compared to controls (*P*=.03). There was no difference in the levels of all other sex hormones between the two groups. The mean estradiol level was also found to be lower in male cases (9.3 pg/mL) as compared to male controls (14.3 pg/mL) (not reported in the tables), but the difference was not statistically significant (*P*=.50), possibly due to the small sample size.

DISCUSSION

Masturbation is considered to be a common normal behavior in adolescents. The physiological and hormonal

Table 1. Characteristic features of events associated with masturbation in 13 Jordanian children.

Patient	Sex	Age of onset	Color change	Posture	Duration	Frequency
1	F	6 mo	Flushing	Prone, supine	5 Min	3/day
2	F	6 mo	Flushing	Knee-chest	2 Min	20/day
3	M	18 mo	Flushing	Prone	5 Min	2/day
4	M	36 mo	Flushing	Prone	5 Min	3/day
5	F	12 mo	Flushing	Friction of thighs	5 Min	5/day
6	M	36 mo	Flushing	Prone	5 Min	2/day
7	F	36 mo	Flushing	Prone	10 Min	2/day
8	F	42 mo	Flushing	Supine	3 Min	3/day
9	F	13 mo	Flushing	Prone	3 Min	3/day
10	F	20 mo	Flushing	Prone	4 Min	4/day
11	F	18 mo	Flushing	Prone	5 Min	6/day
12	F	4 mo	Flushing	Prone, knee-chest	4 Min	10/day
13	F	12 mo	Flushing	Prone	5 Min	3/day

changes that occur during such activity have been well-documented.[5] However, in infants and young children, masturbation can be difficult to recognize due to the absence of genital manipulation, as well as the variable manifestations of this behavior.[3] Childhood masturbation, if unrecognized, may lead to considerable parental anxiety, unnecessary investigations, and inappropriate and potentially harmful therapies.[2-12] The paroxysmal tightening of the thighs, rocking pelvic movements or other rhythmic activities, mechanical pressure applied to the supra-pubic area, grunting, facial flushing, irregular breathing, and sweating during the event, may be misinterpreted as abdominal pain, urinary symptoms, or epileptic seizures.[8-12] In our subjects, masturbation was previously misdiagnosed in many of the cases as a seizure disorder, dystonia, or abdominal pain, resulting in extensive diagnostic testing in the majority of our children, as well as initiation of many unnecessary medications. This demonstrates that even for an experienced movement disorder specialist, the distinction between paroxysmal movement disorders and masturbatory behavior can be difficult to make,[10] if the fact that these infants and young children are responsive to all stimuli during masturbation is missed. In a previous study, 8 of 12 patients with similar characteristics had been treated with different antiepileptic medications.[3]

Our diagnosis was made on the basis of the Fleisher and Morrison study[9] that reported the frequency of an event to vary from 1/week to 12/day, with a mean frequency of 16/week, and a median of 7/week. The mean duration of the event was 9 minutes (median 2.5 minutes, range 30 seconds to 2 hours).[9] The median frequency of events in our study was 4/day, and the median duration of the event was 3.9 minutes. The female-to-male ratio was 3:1 in our study. Varied ratios have been reported in other studies.[6,9,13] Consistent with our findings, masturbation has been reported to start in most children before 2 years of age.[6] Since Jordan is a sexually conservative country with no formal sex education, childhood masturbation may create more parental concern than in Western societies, and the referral rate may differ. Ten (77%) of our children did not attend any follow-up visits after their parents were informed about the diagnosis of childhood masturbation, possibly due to the concern of stigmatization. The etiology of childhood masturbation and its predisposing factors are still controversial and poorly understood. Childhood masturbation has been linked to emotional deprivation, which may in turn lead to more self-stimulation.[14] It may also be associated with sexual abuse.[14] A possible correlation of childhood masturbation to the duration of breast-feeding has also been reported: mas-

Table 2. Hormonal profile of children exhibiting masturbation.

	Status	N	Mean	SD	*P*
Age (years)	Control	13	2.02	0.91	.15
	Case	13	2.67	1.28	
DHEAS (μg/dL)	Control	13	6.59	12.54	.54
	Case	13	4.37	3.43	
17OHP (nmol/L)	Control	13	2.83	2.29	.75
	Case	13	2.55	1.94	
Free testosterone (pg/mL)	Control	13	0.31	0.14	.87
	Case	13	0.31	0.15	
17-estradiol (pg/mL)	Control	13	19.46	8.50	.03
	Case	13	12.31	6.73	
DHEA (ng/mL)	Control	13	1.28	0.97	.89
	Case	13	1.23	0.67	
SHBG (nmol/L)	Control	13	71.43	24.00	.20
	Case	13	86.07	32.27	
Androstenedione (ng/mL)	Control	13	0.14	0.16	.95
	Case	13	0.15	0.19	

Table 3. Hormonal profile of female children exhibiting masturbation, Jordan 2009.

	Status	N	Mean	SD	*P*
Age (years)	Control	10	1.78	0.86	.27
	Case	10	2.34	1.19	
DHEAS (μg/dL)	Control	10	2.27	1.74	.12
	Case	10	4.54	3.80	
17OHP (nmol/L)	Control	10	3.39	2.55	.56
	Case	10	2.77	2.04	
Free testosterone (pg/mL)	Control	10	0.28	0.12	.40
	Case	10	0.34	0.16	
17-estradiol (pg/ml)	Control	10	21.78	6.38	.02
	Case	10	13.20	7.51	
DHEA (ng/ml)	Control	10	0.97	0.30	.24
	Case	10	1.29	0.74	
SHBG (nmol/l)	Control	10	72.88	27.82	.52
	Case	10	82.42	34.54	
Androstenedione (ng/ml)	Control	10	0.16	0.17	.94
	Case	10	0.16	0.21	

turbation was found to be significantly associated with weaning, but not with pacifier usage.[6]

To our knowledge, this is the first study to examine the role of sex hormones in masturbation behavior in children. The finding of a significantly lower level of estradiol in cases as compared to controls is interesting, but should be interpreted with caution. Further studies need to be conducted to conform our results.

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case report

Migration of foreign body from postcricoid region to the subcutaneous tissue of the neck

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Migration of a foreign body from the hypopharynx to the subcutaneous tissue of the neck is a rare event. We report a case of a 48-year-old male who accidentally swallowed a fish bone which was not identified intraoperatively. The patient then presented with migration of the bone to the soft tissue of the neck. We conclude that careful assessment of the patient with a foreign body in the throat is crucial to avoid fatal complications.

Fish bones are the most commonly ingested foreign body. They have a tendency to stick in the pharynx and mucosal layer of the gastrointestinal tract due to their linear shape and sharp edges, and can then cause serious complications if left untreated. In most cases, fish bones can be removed safely by endoscopy, but they may migrate extraluminally to the skin in rare cases.[1] In our case the fish bone was not identified intraoperatively and it migrated from the postcricoid region to the subcutaneous tissue of the neck.

CASE

A 46-year-old male presented to us with odynophagia following ingestion of a fish bone on the same day. Examination of the oral cavity revealed a fish bone stuck in the postcricoid region. An attempt to remove the fish bone under local anesthesia was unsuccessful because the patient had excessive gagging. The patient was then scheduled for direct laryngoscopy and esophagoscopy and removal of the foreign body under general anesthesia. The case was operated on 24 hours later due to heavy demand for emergency operation theater rooms. An ulcerated area was noted intraoperatively in the postcricoid region with no evidence of the embedded fish bone. The patient was discharged on the next day, but two days after the discharge, he developed a swelling in the right side of the neck accompanied by a little pain. He did not seek treatment until eight days later when he noticed the fish bone protruding out of his neck. Examination revealed the fish bone at the lateral border of the neck with no signs of any abscess (**Figure 1**). A CT scan showed the fish bone to be transversely located at the right sternocleidomastoid muscle (**Figure 2**). An emergency exploration of the neck was performed and the fish bone was gently pulled out of the right subcutaneous tissue of the neck (**Figure 3**); it was approximately 1 centimeter in length (**Figure 4**). The patient was discharged on the next day with a seven-day course of antibiotics. On subsequent clinical follow-up, the patient was asymptomatic.

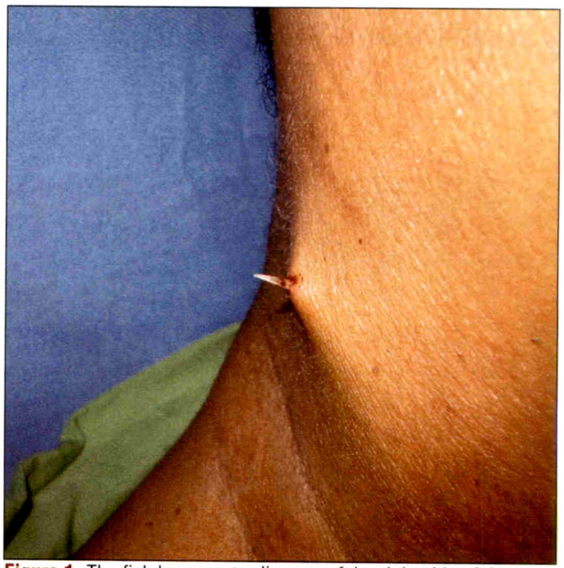

Figure 1. The fish bone protruding out of the right side of the neck.

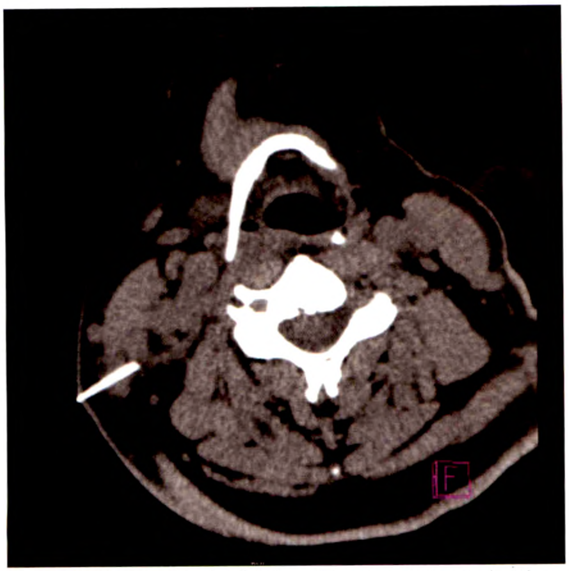

Figure 2. CT revealing the fish bone transversely located at the right sternocleidomastoid muscle.

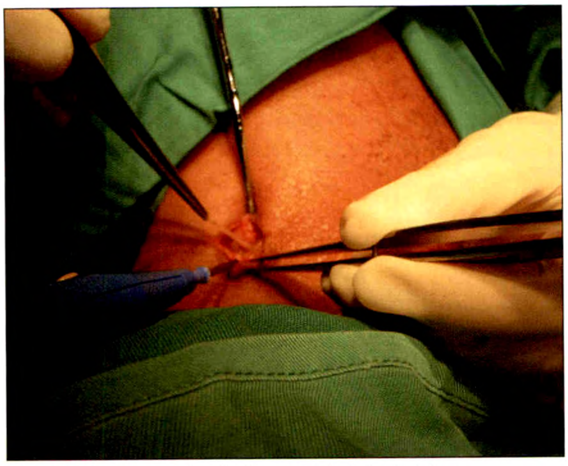

Figure 3. Intraoperative finding revealed the fish bone embedded in the subcutaneous tissue of the neck.

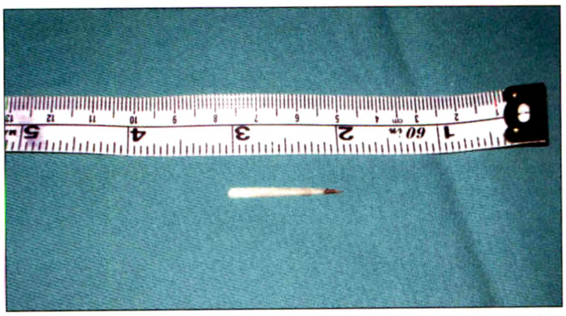

Figure 4. The fish bone after removal, showing linear shape with sharp edge.

DISCUSSION

Ingested foreign bodies are one of the most common problems encountered by medical officers in the casualty ward or the otorhinolaryngology clinic. Most of the time, the foreign body is impacted at the tonsil or the base of tongue.[2] An unidentified foreign body will usually spontaneously pass out through the alimentary tract without any complication. Spontaneous passage is usually related to the size and type of the foreign body.[3] Fish bones are the most commonly ingested foreign body in Asian countries.[1] They have a tendency to stick in the pharynx and mucosal layer of the gastrointestinal tract due to their linear shape and sharp edges, and can then cause serious complications if left untreated.

Migration of ingested foreign bodies is very rare.[1,5] Twenty-four patients were reported with migration of foreign bodies within six years in Singapore.[1] The largest reported series was of 1088 cases of foreign body ingestion in China.[3] Most of the foreign bodies were reported to be lodged in the pharynx, esophagus, stomach, and duodenum. A fish bone has the ability to migrate to the thyroid gland, carotid artery, mediastinum, or subcutaneous tissue due to the constant contraction and relaxation of the pharyngeal musculature, esophageal peristalsis, and surrounding tissue reactions.[6-9]

Once a fish bone is stuck in the throat, it must be removed as soon as possible because of the higher likelihood of its migration due to its linear and sharply pointed contours. In this case, the removal of the foreign body was delayed and the patient was operated on almost 24 hours after the foreign body ingestion. No foreign body could be seen intraoperatively even after putting gentle pressure on the surrounding mucosal layer. We initially thought that the fish bone was dislodged into the gastrointestinal tract, but the patient returned with the fish bone protruding out through the subcutaneous tissue of the neck nine days after the first surgery. Studies have shown that it can take up to 41 days for a fish bone to be excreted naturally from the larynx or the gastrointestinal tract through the skin.[6]

Although plain radiography is a simple and valuable investigation to demonstrate the presence of an opaque foreign body, CT scanning helps to detect the exact site of the impacted foreign body and thus allows for predicting and avoiding any possible complications, especially in the case of migration. In our case, it was necessary to do the CT scan prior to the second surgery to assess the location of the impacted foreign body and its relation to the vital structures of the neck and avoid other possible fatal complications. An extraluminal foreign body has the potential to cause serious complications such as esophageal perforation, periesophageal abscess, retropharyngeal abscess, mediastinitis, arterioesophageal fistula, tracheoesophageal fistula, and carotid rupture. We managed to retrieve the fish bone from the subcutaneous tissue by making an adequate incision in the skin after confirmation of the location of the fish bone.

In conclusion, foreign body ingestion should be managed as soon as possible to avoid fatal and serious complications. It is important to carefully reassess the patient with a foreign body in the throat, especially a fish bone, which has not been found or has been missed by oral or endoscopic examination under general anesthesia.

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case report

Omalizumab therapy in three patients with chronic autoimmune urticaria

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Chronic urticaria is a common skin disease. In about 45% of patients the cause is an autoantibody directed at the α-subunit of the high-affinity IgE receptor (chronic autoimmune urticaria; CAU). Omalizumab is a monoclonal anti-IgE antibody that has a proven role in the treatment of various allergic diseases. We gave omalizumab once every month for 16 weeks to three patients that were refractory to standard treatment, including high doses of antihistamines, leukotriene receptor antagonist, and corticosteroid. There was dramatic improvement in the primary efficacy variable—the change in mean urticaria activity score (UAS) from baseline (i.e., the average over the first 4-week period before omalizumab) to the final 4-week period of omalizumab treatment. There was improvement in the secondary efficacy variables, which included change from baseline in interference with sleep, interference with daily activities, daily diary record of urticaria signs and symptoms based on a scoring system, and rescue medication use. These improvements persisted for 12 weeks after discontinuation of the drug. Omalizumab may have a role in treating refractory cases of CAU.

Urticaria is a common skin disorder that may impair quality of life. Chronic urticaria is defined as the daily or almost daily occurrence of hives for at least 6 weeks. Chronic autoimmune urticaria (CAU) can be found in 45% of such cases, with antibodies against the high-affinity IgE receptor (IgEfcR) or IgE.[1,2] The causal role of IgE in allergic disease is well established.[3] The allergic cascade is initiated when IgE bound to high-affinity FceRI receptors on the surface of basophils and mast cells is cross-linked by allergen, resulting in degranulation of effector cells and release of histamine and leukotrienes. Omalizumab (Xolair) is a recombinant humanized monoclonal anti-IgE antibody approved for the treatment of moderate to severe persistent asthma; it acts by binding to the C3 domain of the heavy chain of IgE.[4] It interrupts the allergic cascade by forming complexes with IgE and down-regulating FceRI as a direct consequence of the reduction in free IgE concentration. By preventing IgE binding to IgEfcR, omalizumab improves patient symptoms and may thus be a novel therapy for CAU.[5]

Treatment of CAU can be challenging for both patients and physicians. Some CAU patients may have a partial or unsatisfactory response to standard therapy, including high doses of antihistamines, leukotriene receptor antagonist, and corticosteroid. We present three cases of CAU that were refractory to standard therapy. All patients received omalizumab for 16 weeks.

CASES

Three patients, aged 24, 39, and 49 years (all females), were diagnosed with CAU. They all had recurrent attacks of urticaria and angioedema affecting the whole body. The urticarial lesions lasted for less than 24 hours. They had no evidence of physical urticaria, urticarial vasculitis, or urticaria secondary to any underlying disease, and there was no history of bronchial asthma, allergic rhinitis, or any other atopic disease. Physical exam was normal and there was no evidence of dermographism. Baseline characteristics and test results are given in **Table 1**. All patients had a positive autologous skin test to serum. This test involves the intradermal injection of 0.05 mL of both sterile autologous serum (ASST) and plasma (APST), with intradermal saline as a negative control, and looking for wheal formation. Serum and plasma samples were centrifuged at 2500 rpm for 5 min and immediately used for the intradermal tests. Readings were taken at 30 minutes. Only an unequivocal wheal-and-flare reaction, with

Table 1. Patient characteristics.

	Patient 1	Patient 2	Patient 3
Age (years)	24	39	49
Sex	F	F	F
Duration of symptoms (years) (continuous)	4	4	5
Dermographism	Negative	Negative	Negative
Total serum IgE (IU/mL)	492.89	300.57	463.72
Anti-thyroglobulin antibody (IU/mL)	55	(+) (343)	2
Anti-microsomal antibody (IU/mL)	(+) (70)	(+) (806)	(+) (55)
Anti-*Helicobacter pylori* antibody (U/mL)	(+) (135)	(+) (280)	(+) (310)
Autologous skin test	(+) to serum	(+) to serum	(+) to serum

(+) positive, titer shown in parentheses. Autoantibody titers were before the start of the therapy with omalizumab

Table 2. Scoring system for urticaria severity.

Score	Pruritis sevirity	Number of hives	Size of largest hive (cm)	Interference with sleep	Interference with daily activities	Erythema severity
0	None	None	None	None	None	None
1	Mild, minimal awareness, easily tolerated	1 to 6	<1.25	Mild, not troublesome, adequate sleep	Mild, not troublesome, little effect on activity	Slight
2	Moderate, definite awareness, bothersome but tolerable	7 to 12	1.25-2.5	Moderate, awoke occasionally, average sleep	Moderate, some interference with activity	Moderate
3	Severe, difficult to tolerate	>12	>2.5	Severe, substantial interference with sleep, poor sleep	Severe, daily activities substantially or completely curtailed	Significant

Table 3. Urticaria activity score (mean) for the three patients at different times after receiving omalizumab.

Week of omalizumab	Patient 1	Patient 2	Patient 3
Week 0	8.52	8.48	7.61
Week 4	4.03	4.26	5.52
Week 8	2.16	2.19	3.35
Week 12	0.61	1.87	1.65
Week 16	0.23	0.26	1.61

$P < .05$ for comparison of week-to-week scores a wheal diameter of at least 1.5 mm more than control, was taken as a positive test result.[6,7] All patients showed a less-than-satisfactory response to maximal doses of antihistamine therapy (hydroxyzine two 25-mg tablets every 6 hours, levocetrizine two 5-mg tablets a day, ranitidine 150 mg twice a day, and montelukast 10 mg orally taken once daily on most days prior to omalizumab therapy. They were kept symptom free on a daily oral corticosteroid for a duration of 2 to 3 months. They refused other steroid-sparing agents such as cyclosporine, methotrexate, or other immunosuppressant medications for fear of side effects. They were gradually weaned off all medications, except the antihistamine, 4 weeks before the start of omalizumab. They were taking 25 to 50 mg of hydroxyzine as often as 4 times a day as needed. After signing an informed consent form, they received omalizumab for 16 weeks. Omalizumab was dosed according to body weight. Serum IgE was obtained 4 weeks before omalizumab treatment was started. Each patient received 300 mg of omalizumab subcutaneously every 4 weeks. All patients maintained a daily diary to record urticaria signs and symptoms based on a scoring system (0-3). Pruritus severity had to be at least moderate (score of 2 on a 0-3 scale), and the urticaria activity score (UAS) (**Table 2**) —a combination of pruritus severity, number of hives, and size of largest hive—had to be at least moderate (minimum score of 4 on a 0-9 scale). At monthly visits, the mean UAS was calculated and rescue medication use was recorded. Mean (standard deviation) UAS declined significantly from baseline to the final 4-week period of omalizumab treatment (8.52 [0.51] to 0.23 [0.42]; $P<.01$) for patient 1 and by a similar degree for the other two patients (**Table 3**). All patients showed complete resolution of symptoms. There was a significant change from baseline in mean score for interference with sleep (from 1.71 (0.46) to 0.00) with $P<.01$ for patient 1, from 2.48 (0.72) at baseline to 0.00 ($P<.01$) for patient 2, and from 2.48 (0.63) at baseline to 0.00 ($P<.01$) for patient 3. All patients had significant improvement from baseline in interference with daily activities, with a decrease in the mean score from baseline in interference with daily activity 2.61 (0.49) to 0.00 ($P<.01$) for patient 1, from 2.61 (0.49) to 0.00 for ($P<.01$) for patient 2, and from 2.48 (0.51) at base line to 0.03 (0.018) ($P<.01$) for patient 3. Treatment with omalizumab resulted in a significant decrease in mean rescue medication use for all patients. The decrease in mean rescue medication for patient 1 was from 4.00 tablets daily at baseline to 0.10 (0.3) tablets during the final 4-week period of omalizumab treatment, from 5.84 (0.37) tablets at baseline to 0.32 (0.54) tablets for patient 2, and from 5.48 (0.51) tablets to 0.39 (0.56) tablets for patient 3. The decrease in mean rescue use of hydroxyzine was significant ($P<.01$) for all patients. They all were followed up for 12 weeks after they had discontinued omalizumab, and there were no reports of symptom recurrence or of other adverse effects.

DISCUSSION

We reasoned that omalizumab, by decreasing circulating IgE levels, would secondarily decrease the IgE receptor density on basophils and cutaneous mast cells and thus prevent activation by autoantibodies. In addition, omalizumab has been reported to ameliorate the symptoms of chronic urticaria in an uncontrolled study of three patients, two of whom also had IgE-mediated asthma.[8-11] An explanation for these observations could be decreased expression of surface IgEfcR due to omalizumab administration.[12,13] In our patients, the mean UAS decreased, the mean rescue medication use declined, and overall therapeutic response and quality of life improved after omalizumab use. This consistency in effect across our three patients, along with outcome measures, suggests that omalizumab may be useful in the treatment of patients with CAU not responsive to antihistamines or other classical therapy. Although none of our patients had asthma, the dose and dosing frequency were based on the total serum IgE and body weight, as is done in asthma patients otherwise eligible for omalizumab therapy. A recent case report suggested that response of CAU to omalizumab therapy was associated with markedly decreased levels of serum donor basophil activation as compared to patients not using omalizumab.[10] Similarly, there are many reports supporting use of omalizumab as an effective therapy for relieving and controlling symptoms of patients with CAU. Kaplan et al[11] recently reported on the beneficial effect of omalizumab in 12 patients with CAU. IgG autoantibodies to the α-subunit of the high-affinity IgE receptor are the cause of the urticaria in many patients with CAU. Kaplan et al proposed that omalizumab may downregulate FceRI expression, preventing mast cell activation by IgG autoantibodies. However, there are reports to the contrary, with patients getting worse with omalizumab therapy. A patient with a dual diagnosis of oral corticosteroid-dependent asthma and CAU received omalizumab for her asthma. Although asthma symptoms improved, this patient, and others, had paradoxical worsening of urticaria immediately after injection of omalizumab.[14,15] Apparently, the presence of detectable autoantibodies to IgEfCR is not required for response to omalizumab, and successful therapy is not invariably associated with decrease in the levels of these autoantibodies when they are present.[8] Patients with CAU can have negative test results for basophil activation and negative results on autologous skin testing.[16]

CAU involves a number of mechanisms in addition to IgEfCR autoantibodies. For example, potential interactions with thyroid autoimmunity and the coagulation pathway may alter mast cell activation.[17] There is a high prevalence of thyroid autoantibodies in patients with CAU.[18] In our series, all patients had high titers of anti-microsomal antibodies, and one had positive anti-thyroglobulin antibody, a finding consistent with the high prevalence of these thyroid antibodies.

The interpretation of the results might have been different had the sample size been bigger or if we had tried to assess long-term prognosis by increasing the period off-treatment to more than 12 weeks. There is no data available at present on the long-term effect of omalizumab in patients with urticaria. Although our experience with omalizumab is limited by the small number of patients, the outcome measures were primarily patient-reported and covered all aspects of subjective assessment of CUA measures.

To summarize, we present three patients with CAU who were refractory to standard treatment but showed good response to treatment with omalizumab. We believe that omalizumab has a role in some patients with CAU and may be useful for treatment of cases not responsive to classical therapy. Further studies should be performed to confirm these findings.

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case report

Congenital lobar emphysema associated with polysplenia syndrome

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Polysplenia, or left isomerism, is a rare heterotaxy syndrome characterized by bilateral bi-lobed lungs, bilateral pulmonary atria, a symmetrical midline liver, and multiple aberrant splenic nodules. We report a case of polysplenia associated with congenital lobar emphysema apart from other typical anomalies. Such an association has not been previously reported. The patient was a young male with progressive exertional breathlessness referred for high resolution CT of the lungs. CT, MRI and echocardiography revealed (in addition to congenital lobar emphysema of right lung) a hemiazygos continuation of the inferior vena cava, a persistent left superior vena cava, multiple splenunculi in the right hypochondrium, midline liver, bilateral bilobed lungs, a large pulmonary artery (suggestive of severe pulmonary artery hypertension) and a large VSD—a typical constellation of findings described in polysplenia syndrome.

Polysplenia or left isomerism is a rare heterotaxy syndrome characterized by bilateral bilobed lungs, bilateral pulmonary atria, a symmetrical midline liver, and multiple aberrant splenic nodules. The spleen is divided into 2 to 16 masses that are located along the greater curvature of the stomach, either in the right or the left quadrant. The cardiovascular anomalies include left-to-right shunts, partial anomalous pulmonary venous return, and interrupted inferior vena cava (IVC) with azygos or hemiazygos continuation.[1] We report a case of polysplenia associated with congenital lobar emphysema apart from other typical anomalies—an association that has not been previously reported in the literature.

CASE

A 40-year-old man presented with insidious-onset progressive breathlessness. Physical examination showed mild central cyanosis, parasternal heave, a short systolic murmur, and a loud second heart sound. A chest radiograph revealed an enlarged pulmonary conus and hilar vessels and a radiolucent area in the right upper zone. High-resolution CT of the chest showed an enlarged main and lobar pulmonary arteries (suggestive of pulmonary arterial hypertension), with an expanded hyperlucent right upper lobe, suggestive of congenital lobar emphysema. The azygous and accessory hemiazygos veins were enlarged, with drainage of the hemiazygos into a persistent left superior vena cava (SVC), which drained into the coronary sinus (**Figure 1**). The liver was seen in the midline position with a short segment of intrahepatic IVC. The stomach was in the right upper quadrant with multiple splenic masses in relation to the greater curvature (**Figure 2**). MRI confirmed the presence of bilateral bilobed lungs with hyparterial bronchi and of the accessory hemiazygos continuation of the IVC (**Figures 3, 4**). Transthoracic and transesophageal echo revealed a large ventricular septal defect (VSD) with pulmonary arterial hypertension, which was suggestive of Eisenmenger syndrome. The patient was referred to a tertiary care cardiothoracic surgery department for further management. To the best of our knowledge this is the first reported case of polysplenia associated with congenital lobar emphysema.

DISCUSSION

The clinical manifestations in polysplenia vary and sometimes can be mild, with many patients (approximately 10%) surviving into mid-adolescence. No single abnormality is pathognomonic for polysplenia; hence, some authors prefer to use the term heterotaxy, and suggest that the different anatomical abnormalities be

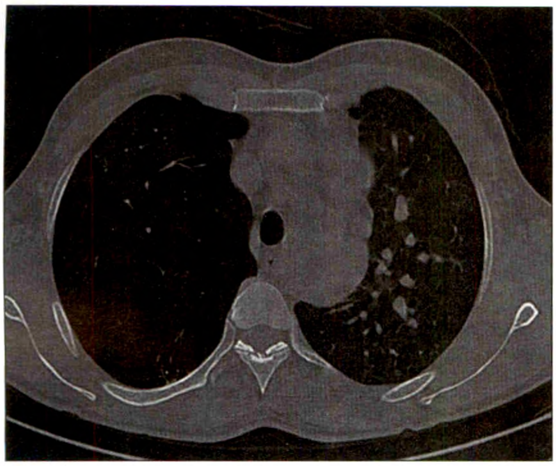

Figure 1. High-resolution CT shows emphysematous right upper lobe with attenuated vascular markings; the accessory hemiazygous is seen draining into the left superior vena cava.

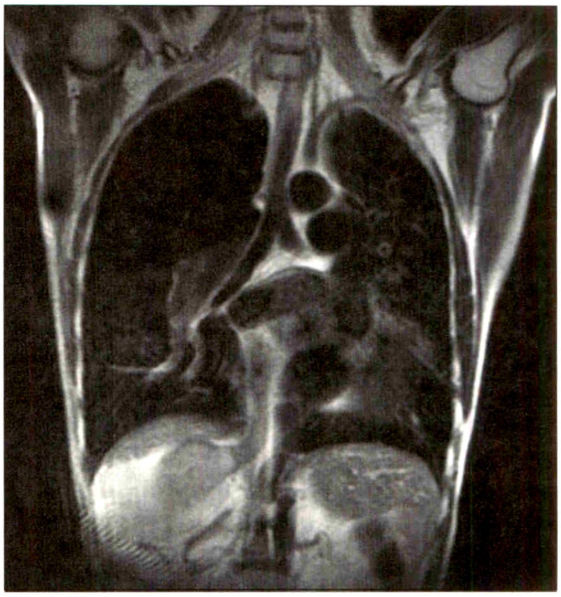

Figure 3. Coronal T2-weighted MRI shows bilateral hyparterial bronchi with emphysematous right upper lobe.

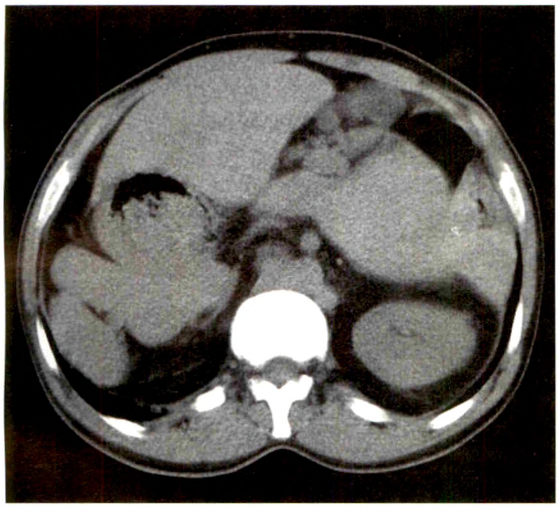

Figure 2. Non-contrast CT of the upper abdomen shows midline liver, stomach on right side of liver and multiple spleens in right hypochondrium.

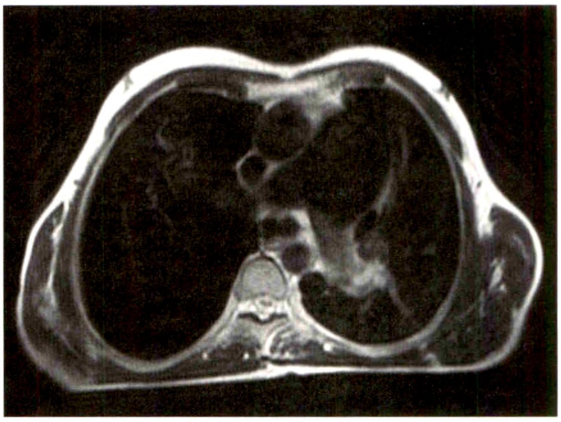

Figure 4. Axial T2-weighted MRI at carinal level shows a grossly enlarged main pulmonary artery, accessory hemiazygous and persistent left superior vena cava.

mentioned while describing a particular patient.[1,2] The various cardiovascular anomalies that may be encountered include atrial septal defect (ASD) (78%), VSD (63%), partial anomalous pulmonary venous return (39%), transposition of the great arteries (31%), right-sided aortic arch (44%), pulmonary valvular stenosis (23%), and subaortic stenosis (8%).[3] In addition, azygous continuation of the IVC is seen in 65% of cases and bilateral SVC in approximately 47%.[1,3]

The abdominal findings may include midline liver (57%), situs inversus (21%), short pancreas, semi-annular pancreas, and preduodenal portal vein.[4-7] Renal agenesis or hypoplasia may also be seen.[4-7] Malrotation, including nonrotation, reverse rotation, and midgut volvulus, is also frequently seen in heterotaxy.[8,9] CT as well as MRI can be used to characterize both the visceral and the cardiovascular anomalies in polysplenia and can provide necessary information for surgical planning.[10] The antenatal diagnosis of cardiosplenic syndromes is possible by sonography supplemented with dedicated fetal echocardiography and color Doppler and, more recently, by ultrafast fetal MRI. Right isomerism is suggested by the presence of complete atrioventricular septal defect, juxtaposition of the IVC and descending aorta, and viscerocardiac heterotaxy. Similarly, in left isomerism, in addition to viscerocardiac heterotaxy and atrioventricular septal defect, azygous continuation of IVC and congenital heart block is also associated.[11]

The pulmonary manifestations include bilateral bilobed lungs and hyparterial bronchi (58%).[1,2] Our patient had a hyperlucent upper lobe with attenuated vascular markings, which was suggestive of congenital lobar emphysema, an entity not described in polysplenia until now. However, the association of congenital heart disease (patent ductus arteriosus, atrial septal defect, ventricular septal defect, total anomalous pulmonary venous return, Tetrology of Fallot with congenital lobar emphysema is well known[12,13] and is reported to occur in 14% to 50% of cases. Both ultrasound and MRI are useful in the antenatal diagnosis of congenital lobar emphysema as well as of other bronchopulmonary malformations. Ultrasound depicts congenital lobar emphysema as a distended fluid-filled anechoic mass that may decrease in size as the gestation progresses. Fetal MRI reveals the high-signal expanded lobe, with compression of the remaining lung and mediastinal deviation (on T2* GRE sequences).[14,15] In our patient, congenital lobar emphysema must have contributed to the worsening of the pulmonary arterial hypertension and dyspnea. The discovery of a bronchopulmonary malformation, including congenital lobar emphysema, on routine antenatal ultrasound should be followed by dedicated fetal echocardiography and a specific search for viscerocardiac heterotaxy.

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case report

Craniopharyngioma in a patient with acromegaly due to a pituitary macroadenoma

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We present the first reported case of a craniopharyngioma as a second primary tumor in a patient with acromegaly due to a growth hormone (GH)-secreting pituitary adenoma. The patient was lost for follow-up for 18 years after trans-sphenoidal pituitary surgery for a GH-secreting pituitary adenoma. She presented with headaches and decreased visual acuity, and showed unsuppressed GH in an oral glucose load test with high IGF-1 levels. Brain MRI showed a suprasellar cystic mass and the patient underwent surgery for cyst drainage resulting in postoperative improvement in her vision. Biopsy of the mass confirmed the diagnosis of a craniopharyngioma. We stress the need for close follow-up of patients with acromegaly with adequate control of GH and IGF-1 levels.

Craniopharyngiomas are rare, mainly sellar/parasellar, epithelial tumors, arising along the path of the craniopharyngeal duct, with an as yet undescribed pathogenesis. Their overall incidence is around 0.13 cases per 100 000 person-years[1] and they account for 2% to 5% of all primary intracranial neoplasms.[2] Growth hormone (GH)-secreting pituitary adenomas are relatively rare, with a prevalence of 50 to 70 cases/million and an incidence of 3 to 4 new cases/million/year.[3-5]

The simultaneous occurrence of both a craniopharyngioma and a GH-secreting pituitary adenoma has not been reported previously in the literature. Cases of the coexistence of craniopharyngioma with prolactinoma[6] or pineocytoma[7] have been previously reported, but this combination is probably merely coincidental. With the rare exception of GH-releasing hormone (RH)-secreting gangliocytomas of the hypothalamus, GHRH-secreting carcinoids,[8-12] and the even rarer GH-secreting islet cell tumors, GH hypersecretion is caused by an intrinsic pituitary neoplasm.

Two theories on the pathogenesis of craniopharyngiomas are that they arise from neoplastic transformation of embryonic squamous cell rests of the involuted craniopharyngeal duct,[13] or that they result from metaplasia of adenohypophyseal cells in the pituitary stalk or gland.[14,15] An interesting and noteworthy fact is that high levels of IGF-1 receptor expression have been demonstrated in cell lines and paraffin-embedded material in a subset of craniopharyngiomas. In fact, in this group of tumors, treatment with an IGF-1 receptor inhibitor caused growth arrest.[16] However, further studies on a larger collection of cases are required to elucidate the clinical value of these results.

Acromegaly is known to be associated with high levels of IGF-1, which through activation of the IGF-1 receptor, results in cell proliferation and growth advantage, whereas the associated IGFBP3 bioactivity promotes an apoptotic advantage.[17-20] Thus, excess GH, by inducing both IGFBP3 and IGF-1 levels, promotes dysregulated cell growth balance characterized by dynamic signals for cell apoptosis versus cell growth advantage. Having a subset of craniopharyngiomas expressing the IGF-1 receptor,[16] but not the IGF-BP3 receptor, suggests that these cells would have a cell growth advantage induced by IGF-1 without the opposing apoptotic effect of IGFBP3, which might have been the case in our patient.

CASE

A 41-year-old woman presented to our university hospital emergency department in 2007 with complaints of occipital headache of increasing intensity and worsening blurred vision in her left eye for the last seven months prior to her visit. Recently, she had also noticed that her menstrual periods were becoming irregular. The

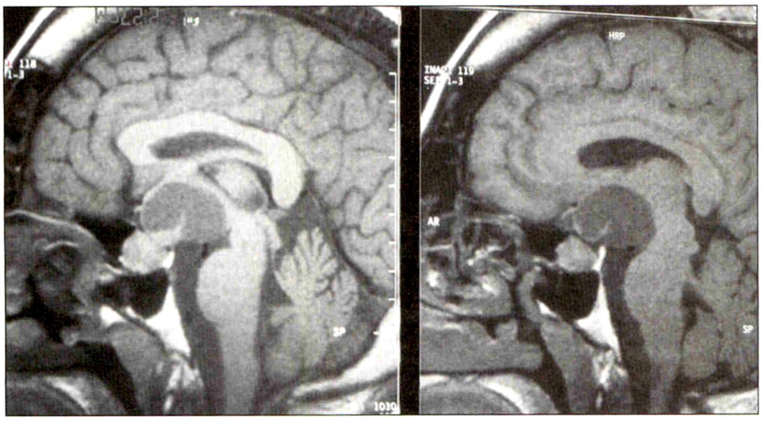

Figure 1. Sagittal MRI brain with enlarged pituitary and suprasellar cyst.

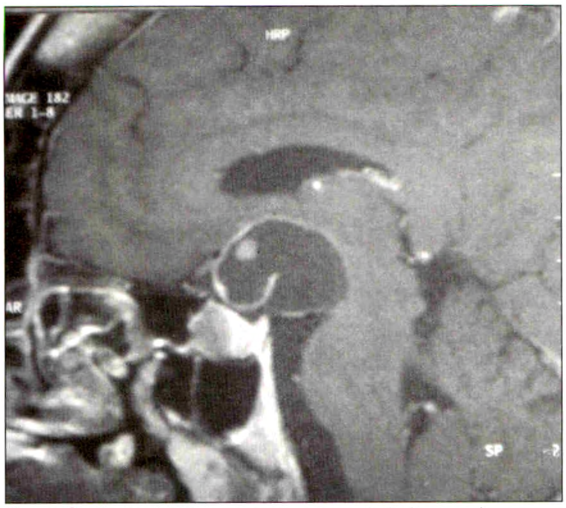

Figure 2. Sagittal MRI brain with enlarged pituitary and suprasellar cyst.

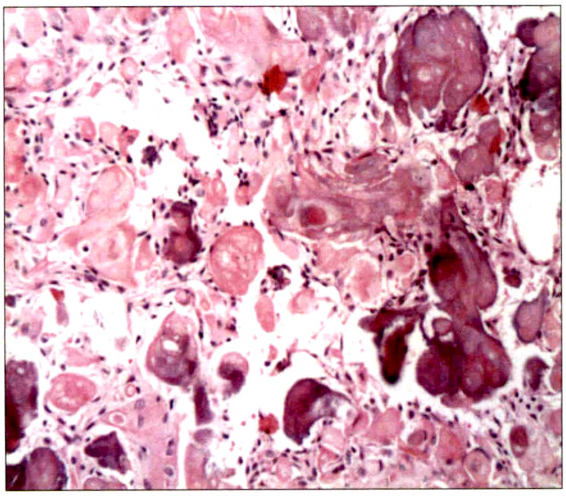

Figure 3. Craniopharyngioma anastomosing epithelial islands with palisaded layer of cells and a center of stellate cells along with nests of keratin (Hematoxylin and eosin, ×20)

patient had a history of acromegaly treated with transsphenoidal resection of a pituitary tumor in 1989. She was also known to have primary infertility for which she had not sought any medical help. She had been lost to follow-up since her surgery until this visit. On examination, she was found to have acromegalic features and a left eye temporal visual field defect on confrontation and visual field mapping. An x-ray of her skull revealed thickened calvaria with a wide, double-floored sella. An MRI of her brain revealed a multiloculated cystic mass seen in the suprasellar region with extension more to the left side, causing a mass effect on the surrounding structures without midline shift or hydrocephalus (**Figures 1 and 2**).

In the two months since her first visit, the patient had noticed a significant decrease in her left eye vision with an ability only to perceive light. On examination, her right eye was found to have a new temporal visual field defect. At that time, endocrine testing revealed hypogonadotropic hypogonadism with a serum prolactin level that was mildly elevated at 45 ng/mL. She was also found to have central hypothyroidism. Her GH was not suppressed even after an oral glucose load test. The baseline GH was 17.3 ng/mL; it became 13.0 ng/mL and 10.3 ng/mL at one and two hours. The IGF-1 level of 1006 ng/mL along with the unsuppressed GH confirmed the previous diagnosis of acromegaly. She underwent a left pterional craniotomy with subfrontal approach under microscopic guidance to obtain a tissue diagnosis and drain the cystic tumor. She developed transient diabetes insipidus postoperatively, but her vision improved significantly following surgery. She received perioperative stress doses of steroids, which were tapered rapidly afterwards. The patient was also started on L-thyroxine replacement therapy. Tissues obtained from surgery confirmed the diagnosis of a craniopharyngioma (**Figure 3**). Six months after presentation, she was still being followed up at the endocrine and the neurosurgery clinics for further management.

DISCUSSION

To our knowledge, this is the first reported case of a craniopharyngioma developing in a patient with a GH-secreting pituitary adenoma. A craniopharyngioma comprises only 3% of intracranial neoplasms with a tendency to affect children and young adults.[21] Headache and visual field defects are the most common presenting clinical features (64% and 55%, respectively). Craniopharyngiomas can result in a high incidence of deficiencies of GH, luteinizing hormone/follicle-stimulating hormone, thyroid-stimulating hormone, and adrenocorticotropic hormone (>80%), and diabetes insipidus (65%).[22]

Both a craniopharyngioma and the pituitary gland share the same embryonic origin. They arise from the Rathke pouch, which is a diverticulum of the roof of the embryonic oral cavity.[23] A developmental mishap or a genetic abnormality might explain the presence of these two different tumors in the same individual. A previous study attempted to implicate the beta-catenin gene in the initiation and subsequent growth of craniopharyngiomas and pituitary adenomas. Several mutations of the beta-catenin gene have been found in adamantinomatous craniopharyngiomas, but not in pituitary adenomas.[24]

The collision lesions of the sellar region (two simultaneous tumors or cysts in the perisellar region) have been previously described in case reports or small case series. The most common presentation of collision sellar lesions is the presence of a simultaneous pituitary adenoma with a Rathke cyst. A previous case report exists of simultaneous GH-secreting pituitary microadenoma with a Rathke cleft cyst, causing compression of the optic chiasm with resultant visual field defects.[25] Adenomas in this situation usually secrete prolactin.[26] This is probably due to stalk compression from the cyst with resultant lactotroph hyperplasia. There is one case report of a craniopharyngioma with a gonadotrophin-secreting pituitary adenoma, but this occurrence remains very rare.[27]

In a study of the nationwide Swedish cancer family registry, meningioma was reported as the most frequent second primary tumor in individuals with a previous diagnosis of a first primary tumor of pituitary or parathyroid adenomas.[28] In a group of patients with pituitary adenomas who underwent surgery and radiotherapy, meningioma was the most common second primary intracranial neoplasm at 10 to 20 years of follow-up.[29] Craniopharyngioma was not reported in this series of more than 400 patients. Few case reports exist of simultaneous pituitary adenomas and meningiomas, either in the parasellar region or the fourth ventricle without any previous history of radiotherapy.[30,31]

Acromegaly has been arguably associated with an increased risk for several tumors with the strongest association reported with colorectal carcinoma.[32] Increasing evidence from in vitro data, animal studies, and studies in nonacromegalic patients continue to strengthen the role of the GH/IGF-1 axis in tumor development. In addition to the classical endocrine actions of the GH/IGF-I axis, important paracrine and autocrine effects of locally produced GH and/or IGF-I are being increasingly recognized.[33]

Our patient had evidently suffered from uncontrolled acromegaly for several years with elevated GH and IGF-1 levels. Given the close anatomic proximity of the GH-secreting pituitary macroadenoma and the suprasellar craniopharyngioma in this patient, it would be reasonable to assume that the paracrine effects of the GH/IGF-1 axis would have been exaggerated. There is a paucity of literature investigating the tumorigenic effects of the GH/IGF-1 axis on brain tumors and craniophayngiomas, in particular, due to the rarity of this occurrence. Though rare, this complication of acromegaly might have been potentially prevented or detected earlier if the patient had maintained closer follow-up after her trans-sphenoidal pituitary resection. Better biochemical control of her acromegaly might have prevented the emergence of the craniopharyngioma. It is debatable whether the patient's clinical picture and biochemical evidence of acromegaly were the result of GHRH secretion from the suprasellar craniopharyngioma. A possible scenario is that she had a craniopharyngioma at time of initial presentation, which was too small to be detected by the radiological studies done at that point of time. However, we have no proof that this was the case. Additionally, if the GH excess was the result of GHRH, it would have resulted in pituitary GH-secreting cell hyperplasia, rather than a distinct pituitary adenoma, which is the case in our patient. She first underwent trans-sphenoidal surgery for the GH-secreting pituitary adenoma, which did not result in adequate control of her disease. After an interval of several years without follow-up, she presented with the second pathology. It is worth noting that in a series of 121 cases of craniopharyngioma in children, none presented with acromegaly. In fact, craniopharyngioma mostly presents with hormone deficiency syndromes, with the sole exception of the expected elevation in serum prolactin levels.[22] In fact, there were no previous reported cases of any GHRH-secreting craniopharyngioma in a Medline search. Unfortunately, we were not able to obtain serum GHRH levels in this patient due to the unavailability of this assay in any of the local hospitals and laboratories.

In summary, we report a patient with uncontrolled acromegaly who presented with headache and visual field defects due to a suprasellar craniopharyngioma. She had central hypothyroidism and hypogonadotrophic hypogonadism. To our knowledge, this is the first reported case in the literature.

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case report

Prenatal diagnosis and molecular cytogenetic analysis of a *de novo* isodicentric chromosome 18

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Isodicentric chromosome 18 [idic(18)] is rare structural aberration. We report on a prenatal case described by conventional and molecular cytogenetic analyses. The sonography at 24 weeks of gestation revealed multiple fetal anomalies; radial aplasia and ventricular septal defect were significant features. Routine karyotyping showed a derivative chromosome replacing one normal chromosome 18. The parental karyotypes were normal, indicating that the derivative chromosome was *de novo*. Array comparative genomic hybridization (array-CGH) revealed 18p11.21→qter duplication and 18p11.21→pter deletion for genomic DNA of the fetus. The breakpoint was located at 18p11.21 (between 12 104 527 bp and 12 145 199 bp from the telomere of 18p). Thus, the derivative chromosome was ascertained as idic(18)(qter→p11.21::p11.21→qter). Fluorescent in situ hybridization (FISH) confirmed that the derivative chromosome was idic(18). Our report describes a rare isodicentric chromosome 18 and demonstrates that array-CGH is a useful complementary tool to cytogenetic analysis for reliable identifying derivative chromosome.

Trisomy 18 is a common chromosomal disorder resulting in well-known dysmorphic features and organ malformations. Isochromosome 18 [i(18q) or i(18p)], however, is rare cytogenetic aberration. Because the dicentric pattern is difficult to establish using conventional banding analysis alone, the report of isodicentric chromosome 18 [idic(18)] is more rare.[1] We present a fetus with a previously undescribed isodicentric chromosome 18 [idic(18)(qter→p11.21::p11.21→qter)] characterized by array comparative genomic hybridization (array-CGH) and fluorescent in situ hybridization (FISH).

CASE

A 28-year-old gravida 3 para 0 Chinese woman was referred to our hospital at 24 weeks of gestation because sonography showed multiple fetal anomalies, including bilateral absent radii and short humeri, deformed hands, a right cross-foot, a ventricular septal defect, bilateral choroid plexus cysts, a thickened neck and nape soft tissue. The pregnancy was uneventful. The woman and her husband were healthy and non-consanguineous. There was no family history of congenital malformations. She denied any exposure to alcohol, teratogenic agents, irradiation or infectious diseases during the pregnancy. The analysis of cultured amniotic fluid cells yielded 100% of metaphases with chromosomal abnormality involving chromosome 18. The parental karyotypes were normal. Based on the sonographic findings and the unbalanced chromosomal aberration, the pregnancy was terminated at 27 weeks of gestation. At termination, the fetus was phenotypically male measuring 32 cm in length. Physical examination revealed bilateral valgus palms, bilateral short upper limbs, right cross-foot, and left pes valgus (**Figure 1**). Necropsy further confirmed bilateral absent radii, bilateral ulnas length 2.5 cm, left humerus length 2.0 cm, right humerus length 3.0 cm, bilateral deformed articulatio capitis humeri, articulatio cubiti, articulatio carpi, articular genu and articulatio talocruralis, angio-

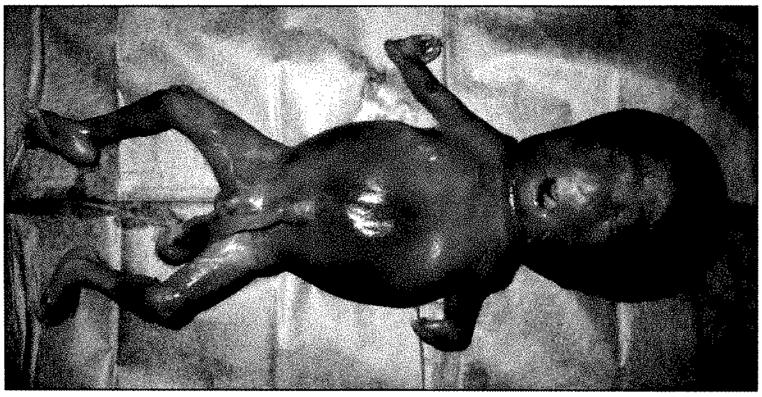

Figure 1. Photograph of the fetus after termination at 27 weeks of gestation.

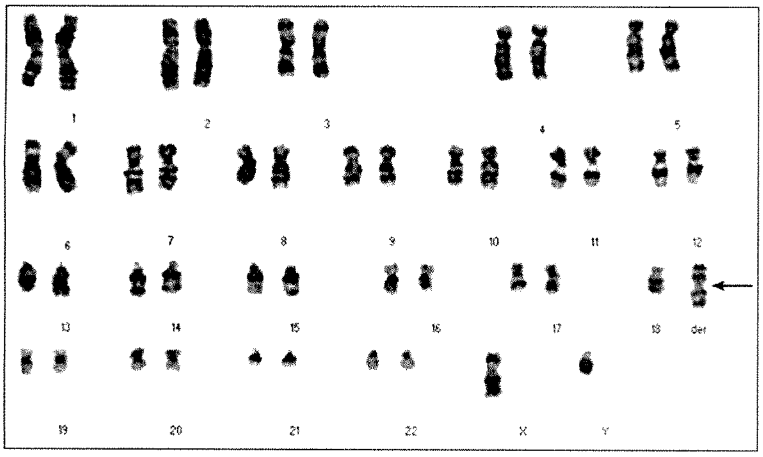

Figure 2. G-banded karyotype of the proband.

cavernoma in the cerebellum, ventricular septal defect, aorta diameter 0.4 cm, and pulmonary artery diameter 0.3 cm. Other internal organs seemed normal on gross appearance.

G-banding analysis on cultured amniotic cells showed that one chromosome 18 was replaced by a derivative chromosome (**Figure 2**). The origin and architecture of the derivative chromosome were unclear. Both parents had normal karyotypes, indicating a *de novo* origin of the derivative chromosome 18. Array-CGH revealed 18p11.21→qter duplication and 18p11.21→pter deletion for genomic DNA of the fetus, the breakpoint was located at 18p11.21 (between 12 104 527 bp and 12 145 199 bp from the telomere of 18p), which indicated that the derivative chromosome was idic(18)(qter→p11.21::p11.21→qter) (**Figure 3**). FISH analysis using a satellite centromere-specific probe for chromosome 18 (D18Z1), whole chromosome painting probe for chromosome 18 (WCP18), 18q/18p telomere-specific probes (D18S1390 and D18S552) (Cytocell, UK) confirmed that the derivative chromosome 18 was idic(18) (**Figure 4A to 4D**). Written informed consent was obtained from the patient's parents. This study was performed according to the guidelines of Shenzhen People's Hospital, which abides by the Helsinki Declaration on ethical principles for medical research involving human subjects.

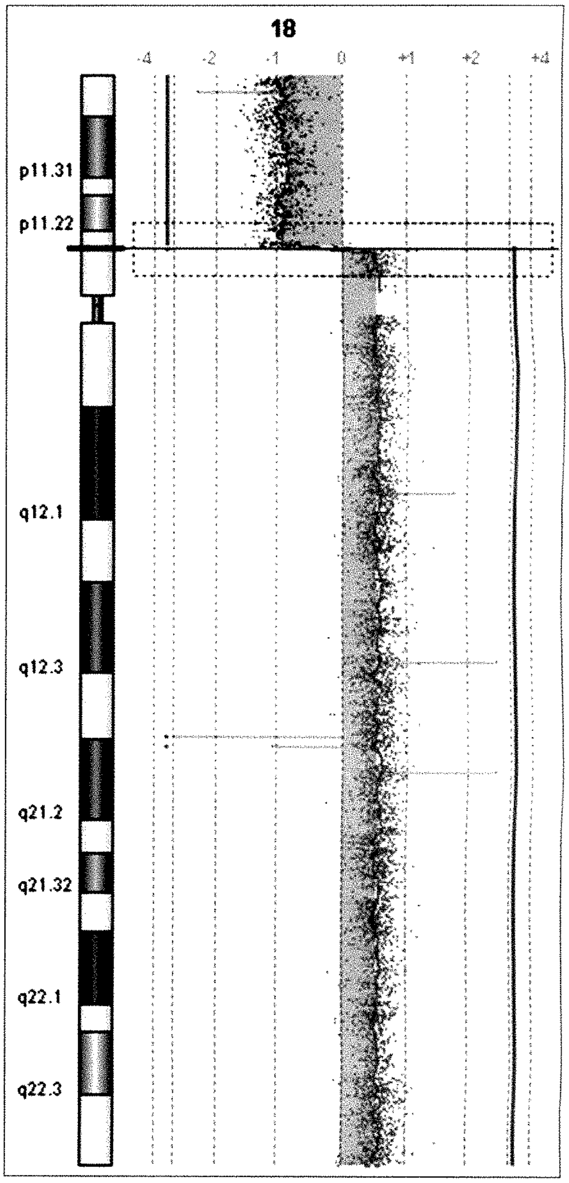

Figure 3. Array comparative genomic hybridization ratio profile of the derivative chromosome 18.

DISCUSSION

The current standard technique for detecting chromosomal abnormalities involves metaphase analysis of cultured cells. Due to limited resolution, only large aberrations (> 3 Mb) and translocations can be identified by conventional cytogenetic techniques. Therefore, in this case, G-banding analysis could not identify the origin and architecture of the derivative chromosome. Only by applying array-CGH could the derivative chromosome be ascertained to be idic(18)(qter→p11.21::p11.21→qter), indicating that array-CGH is a useful tool for describing derivative chromosome.

Isochromosomes are supernumerary marker chromosomes made up of two copies of the same arm of a chromosome containing 1 to 2 centromeres, so that they form a mirror image of each other.[2] Dicentric autosomes are not common in humans because they often result in partial trisomy and partial monosomy status. There are two mechanisms by which an isodicentric chromosome can be formed. One is an isolocal U-shape sister chromatid exchange (isolocal break in two chromatids and rejoining of broken ends) in either the S or G2 phase.[3] The other is a chromosomal deletion during the G1 phase and U-shape rejoining of the broken ends of the chromatid after replication of the deleted chromosome.[4] Both mechanisms will result in a symmetrical isodicentric chromosome. Recently, a new type of asymmetrical pseudoisodicentric chromosome derived from non-isolocal sister chromatid breaks of a parental homolog has been reported.[5]

It has been noted that numerical and structural abnormalities of chromosome 18 usually produce severe phenotypic abnormalities. The isochromosome 18q [i(18q)] often demonstrates characteristics of pure trisomy 18 owing to three copies of 18q as well as monosomy 18p due to missing one copy of 18p. The typical phenotype of i(18q) is very comparable to complete trisomy 18 and includes severe growth retardation, occipital prominence, low set ears, downward-slanting palpebral fissures, overlapping flexed fingers, rocker-bottom feet, congenital heart defects, and skeletal abnormalities. The monosomy 18p (18p-) often results in psychomotor retardation, short webbed neck, low posterior hairline, hypospadias, male genital anomalies and holoprosencephaly.[6,7] The present fetus who had 18p11.21→qter duplication and 18p11.21→pter deletion demonstrated typical features of trisomy 18, including ventricular septal defect, limbs malformations and skeletal abnormalities. However, the cardinal features of 18p- were not present. This may be because 18p- features are less specific and camouflaged by the more drastic manifestations of trisomy 18q or a significant amount of short arm material (18p10→p11.21) is present. Furthermore, patients with a monocentric i(18q) usually have manifestations of both trisomy 18 and the 18p- syndrome; however, if the isochromosome 18 is dicentric, only the characteristics of trisomy 18 are likely to be exhibited.[8] Therefore, the redundant centromere presented in our case might influence the presence of phenotypes of 18p-. Wulfsberg et al suggested that Turner-like features associated with 18p- may be determined by monosomy for 18p11.[9] Our case, in whom 18p10→p11.21 is present, lacked Turner-like features, which supports the conclusion of Wulfsberg et al, and we postulate that 18p10→p11.21 may be the critical region for Turner-like features associated with 18p-. Our case had severe limb malformation. A dosage imbalance of SALL3 located on 18q may be responsible for the radial defects.[10] Our case also has angiocavernoma in the cerebellum, which to our knowledge, has not been reported in cases with idic(18) or i(18q).

In conclusion, this report describes a rare *de novo* isodicentric chromosome 18 and demonstrates that

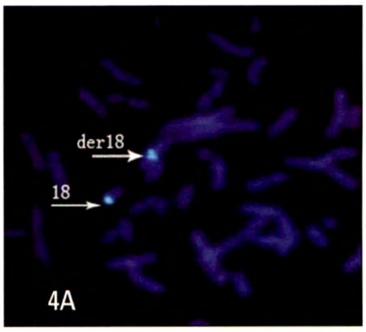

Figure 4A. A partial metaphase spread hybridized with a chromosome 18 centromere probe (green) showing two signals in derivative chromosome 18 and one signal in normal chromosome 18.

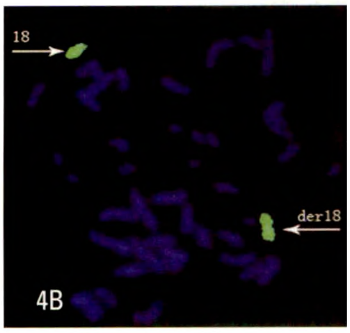

Figure 4B. A metaphase spread hybridized with a whole chromosome 18 library (green) identifying the derivative chromosome as being composed of chromosome 18 material.

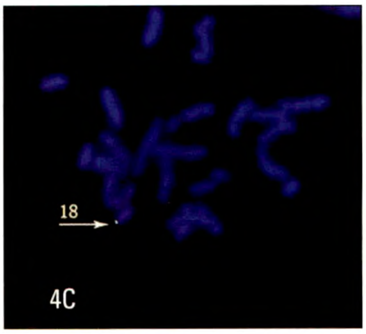

Figure 4C. A metaphase spread hybridized with 18p subtelomere probe (green) showing only one normal chromosome 18 with a p-telomere signal at one end.

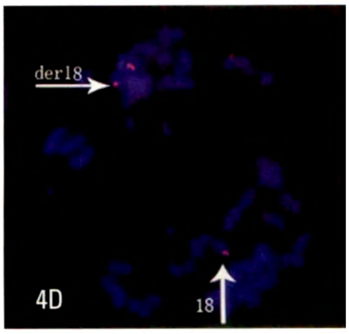

Figure 4D. A metaphase spread hybridized with 18q subtelomere probe (orange) showing one derivative chromosome 18 with q-telomere signals at both ends and one normal chromoson 18 with q-telomere signal at one end.

array-CGH is a useful complementary tool for cytogenetic analysis in reliably describing a derivative chromosome. There might still be some technical and financial problems using the arrays in routine diagnostics, but array-CGH has already become an important tool for the diagnosis of genetic disease in clinical practice.

Acknowledgments

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RE: Combined serological tests for pulmonary aspergillosis

To the Editor: I read the recent publication by Gao et al on combined serological tests for pulmonary aspergillosis with a great interest.[1] Gao et al concluded that "Combining two serological tests increased the specificity of diagnosis, but further trials are needed to prove the value of this approach."[1] First, clarification of the quality control process for the serological test in this study is necessary since this can affect interpretation of the results. In addition, I would like to add some ideas on this report. In addition to a trial on the diagnostic properties in other settings, a study on the cost effectiveness of this new approach should also be conducted. Also, there should be a diagnostic protocol for management of cases with controversial and discordant results from the combined serological tests. Indeed, the problem of cross reaction to other nonpathological *Aspergillus* spp should also be kept in mind when one uses the serological test for diagnosis.[2] However, Lau et al suggested that "Notwithstanding the advantages of molecular tests, serological assays remain clinically useful for patient management."[3]

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Reply

We appreciate the comments of Dr. Wiwanitkit on our article. All procedures for the serological test in this study were performed according to the *Guideline for Internal Quality Control for Quantitative Measurements in Clinical Laboratories of China*. The primary aim of this pilot study was to investigate the approximate incidence of invasive pulmonary aspergillosis in patients with acute exacerbation of COPD and the diagnostic value of combined serological tests, so further analysis on cost-effectiveness and diagnostic protocol is limited by the relatively small number of confirmed cases. We think that the possible solution to this problem is large multicenter clinical trial conducted in high-risk population.

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RE: Water pipe (shisha) smoking among male students of medical colleges in the eastern region of Saudi Arabia

To the Editor: I read the article 'Water pipe (shisha) smoking among male students of medical colleges in the eastern region of Saudi Arabia.'[1] by Taha et al with interest. I applaud the authors for conducting such study and would like to share my views on the same.

The study reported that significantly higher proportion of students were from the College of Medicine as compared to the other two. However, this statement can be misinterpreted since a quarter of students from the College of Dentistry (29.41%, 15 out of 51) were 'shisha' smokers as compared to the other two groups namely, College of Medicine 8.44% (19 out of 225) and College of Applied Medical Sciences (13.68%, 13 out of 95). It would have been interesting to know whether any attempt was done to conceal the identity of the students while questionnaires were filled since it could have made significant impact on the outcome of the study involving such a sensitive issue. Nonetheless, I really appreciate the efforts of authors to conduct such a study.

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PMID: 21060162
DOI: 10.4103/0256-4947.72276

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Reply

We would like to thank Dr. Aggarwal for his comments about the article. Dr. Aggarwal was right in stating that a greater percentage of students from the College of Dentistry were 'shisha' smokers as compared to other two groups. The misinterpretation could be due to the fact that we used a column total instead of row total as a denominator in Table 3. We realized this when constructing Table 3 (that a high prevalence of shisha use is seen among dental students and least among the medical students). However, our aim was to highlight the proportion of shisha smokers in each college in relation to the total number of students who smoke. However, the use of either column total or row total will not change the result of the statistical association. Yes, the identity of the students was concealed while questionnaires were filled. No names appeared on the questionnaires. This is an essential ethical consideration in conducting such a study.

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RE: Sheehan syndrome with reversible dilated cardiomyopaty

To the Editor: I read the case report on 'Sheehan syndrome with reversible dilated cardiomyopaty'[1] by Laway et al with interest. The case indeed looks interesting. However, it could also have been a case of peripartum cardiomyopathy, which can have an onset up to 5 months after pregnancy[2] and in most cases, recovery of left ventricular function occurs within 6 months of its diagnosis.[2,3] Also, a previous history of subclinical hypothyroidism cannot be ruled out, which might have been precipitated by the peripartum events. The authors also failed to mention the details of antitubercular therapy, which could have altered the course of disease and contributed to drug-induced dilated cardioyopathy.

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DOI: 10.4103/0256-4947.72278

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3. Elkayam U, Akhter MW, Singh H, Khan S, Bitar F, Hameed A, Shotan A. Pregnancy-associated cardiomyopathy: clinical characteristics and a comparison between early and late presentation. Circulation 2005; 111:2050

Reply

In response to the comments by Dr. Aggarwal, our patient had a last childbirth two years before and was not symptomatic during these two years, so the chance of it being due to peripartum cardiomyopathy does not arise. We do not agree with the author that the patient could have subclinical hypothyroidism precipitated by peripartum events. The patient did not have subclinical hypothyroidism (where we expect a normal T4 and mild elevation of TSH); in fact, she had severe secondary hypothyroidism as a part of panhypopituitarism because of Sheehan syndrome. Regarding details of antitubercular treatment, the patient was put on category 1 treatment consisting of isoniazid, rifampicin, pyrazinamide and ethambutol in recommended dosages. Yes, we believe that secondary adrenal insufficiency was precipitated by rifampicin, but we do not think cardiomyopathy was precipitated by antitubercular treatment, considering the known side effect profile of these drugs.

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The prognostic importance of cystatin C in severe systolic dysfunction without chronic kidney disease

To the Editor: Cystatin C has been identified as a novel biomarker that is more sensitive in detecting early kidney dysfunction. We aimed to investigate the prognostic importance of cystatin C in stable heart failure patients who had an ejection fraction (EF) of <35% and a glomerular filtration rate (GFR) of >60 mL/min/ 1.73 m^2. Seventy-five stable heart failure patients (50 males and 25 females with a mean age (SD) of 67.6 (10.6) were included in this study. All patients were evaluated using Doppler echocardiography, and biochemical variables including measurement of cystatin C were measured at baseline. Patients were prospectively followed-up for 13(1) months. The endpoints were all-cause mortality and major cardiac adverse events (MACE), which were mortality and rehospitalization. Cystatin C serum levels were measured using a sandwich enzyme immunoassay (ELISA) kit (Biovendor Research and Diagnostic Products, www.biovendor.com).

Serum levels of cystatin C ranged from 0.47 to 2.02 ng/mL (median 1.34 [0.42] ng/mL). Eleven patients died and 34 MACE occurred during follow-up. Mean cystatin C level was significantly higher in the participants who died or had MACE. Cystatin C was significantly correlated with serum creatinine level (*P*=.001, r=0.368) and NYHA class (*P*=.02, r=0.25). Mean high-sensitivity C reactive protein (hsCRP, NYHA class, uric acid and troponin I were significantly higher and hemoglobin level was lower in the mortality group (**Table 1**). Creatinine and GFR were similar between the patients who survived or had MACE. Echocardiographic parameters and biochemical variables (except total cholesterol) were not statistically different. Logistic regression analysis revealed that only cystatin C level was an independent risk factor for MACE rate (hazard ratio=32.56, 95% confidence interval, 2.26-468.62, *P*=.01). For mortality rate, the same analyses revealed an independent risk with cystatin C, which did not reach significance (OR: 37.1, 95% CI, 0.94-1464, *P*=.054). According to ROC analysis, cystatin C levels >1.55 ng/mL could predict mortality with 82% sensitivity and 73% specificity. Furthermore, cystatin C levels >1.45 ng/mL could predict MACE rate with 76% sensitivity and 83% specificity. Kaplan-Meier analysis showed that patients who had serum cystatin C levels of >1.5 ng/mL had a significantly higher total death rate compared with lower levels (*P*<.001).

Table 1. Clinical characteristics of survivors and patients who were free of major cardiac adverse events (MACE) and characteristics of patients who died or had MACE during follow up.

	Survivors (n=64)	Mortality group (n=11)	*P*
Age (year)	67.2 (10.8)	70.0 (9.4)	.79
NYHA class III	16	6	.047
Ejection fraction	29.0 (5.6)	30.5 (6.4)	.31
hsCRP (mg/dl)	1.09 (1.11)	3.89 (3.18)	.03
Hemoglobin (g/dl)	13.0 (1.6)	11.6 (1.8)	.001
GFR (mL/min per 1.73 m^2)	-	-	
Creatinine (mg/dl)	0.91 (0.22)	1.04 (0.17)	.075
Cystatin C (ng/mL)	1.27 (0.41)	1.71 (0.21)	.016
Uric acid (mg/dl)	6.0 (1.9)	7.8 (2.2)	.009
sTroponin levels (µg/dl)	0.08 (0.27)	0.44 (0.88)	.02
	MACE free (n=41)	**MACE (n=34)**	***P***
Hemoglobin (g/dl)	13.3 (1.6)	12.3 (1.7)	.01
GFR (mL/min per 1.73 m^2)	79.4 (17.0)	81.7 (22.2)	.63
Cystatin C (ng/mL)	1.15 (0.37)	1.55 (0.35)	<.001
High sensitive CRP (mg/dl)	0.98 (0.87)	2.1 (2.45)	.023
Troponin I (µg/dl)	0.031 (0.17)	0.29 (0.62)	.02

Values are mean (standard deviation).

Cystatin C is a small serine protease inhibitor that is secreted from almost all active cells in the body. It has been identified as a novel biomarker that is more sensitive in detecting early kidney dysfunction compared with creatinine and creatinine-based estimated GFR.[1] Previous studies dealing with the prognostic value of cystain C in heart failure were conducted on patients with EF>40%.[1,2] Our results showed further insight about the prognostic value of cystatin C in stable heart failure patients who had lower ejection fraction (<35%). The results of the Cardiovascular Health Study showed that elevated cystatin C concentrations were associated with incrementally increasing mortality risk, particularly over 1.0 ng/mL.[3] The results of the study confirm the same result since none of the patients in our study group having a baseline cystatin C level of < 1 ng/mL died during follow-up.

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images

Dysparathyroidism: A Clinical Window

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Calcifications in various tissues are a feature of both hyper- and hypocalcemic states. A 13-year-old girl presented with recurrent episodes of generalized tonic-clonic seizures for the previous three months. She was in the sixth standard with a history of learning difficulties. There was also a history of delayed milestones of development. On examination, the patient had a height of 123 cm (<3rd percentile), with an upper segment of 63 centimeters, weight 24 kg (<3rd percentile) and normal facies. There were bilateral short ring fingers (**Figures 1 and 2**), and short 3rd and 4th toes. Neurological examination showed a low IQ rating. Complete hemogram, urea, creatinine, liver function tests, chest x-ray , serum sodium, potassium and USG abdomen were normal. The EEG showed marked sharp wave activity while the CT scan of the brain revealed bilateral basal ganglia as well as parietal lobe calcifications (**Figure 3**). Serum calcium, phosphate and magnesium were 6.4 mg/dL (normal, 8-10 mg/dL), 7 mg/dL (normal, 2.5-5 mg/dL) and 2.5 mg/dL, respectively. The serum 25-hydroxy cholecalciferol level was 47 ng/mL (normal, 15-80 ng/mL) while serum PTH level was 450 pg/mL (normal, 15-65 pg/mL). X-ray of the kidney showed multiple bilateral stones (**Figure 4**).

This was a case of pseudohypoparathyroidism with features of Albright osteodystrophy and other trophic hormone resistance as well. Common sites involved are

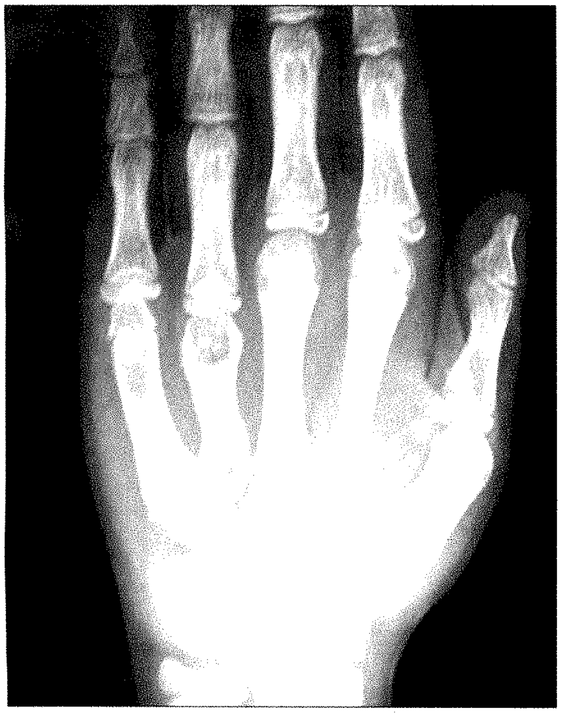

Figure 1. Photograph shows X-ray of hand AP view with short fourth metacarpal bone.

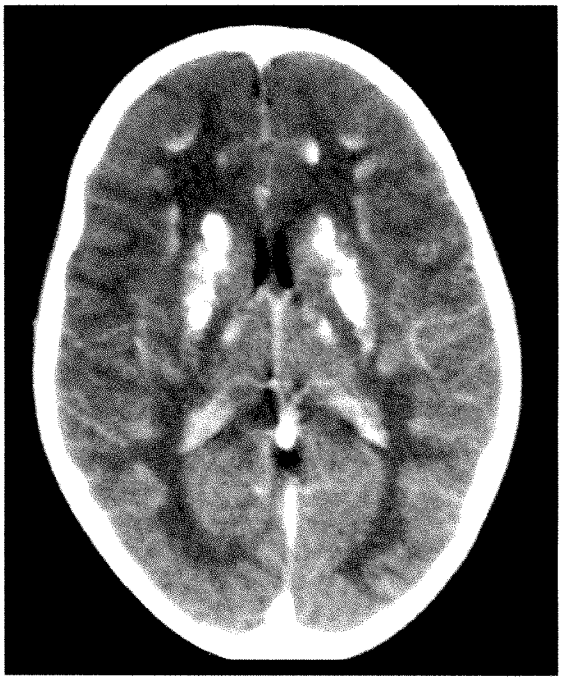

Figure 2. Photograph shows CT Scan of brain (plain) revealing bilateral basal ganglia calcification.

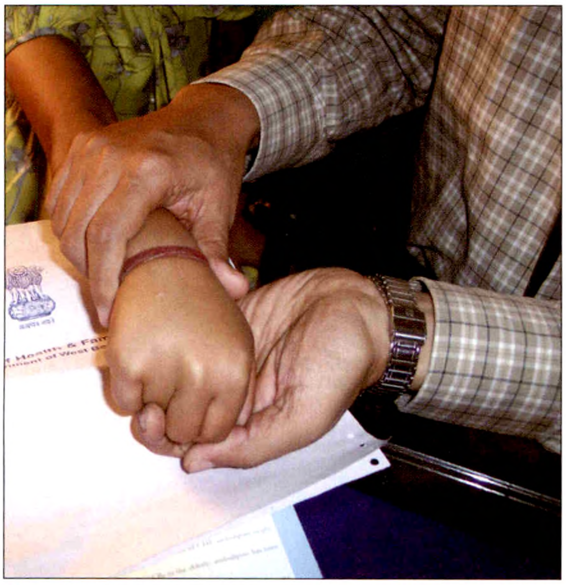

Figure 3. Photograph shows short ring finger of right hand.

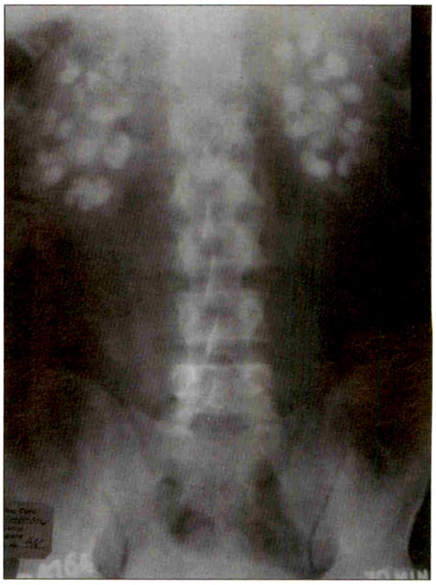

Figure 4. Photograph shows straight X-ray of abdomen with bilateral extensive nephrocalcinosis.

walls of the blood vessels, periarticular areas, skin, soft tissues and viscera, notably lung, heart, kidney, intestine and brain. Although phosphate is initially low in primary hyperparathyroidism, progressive renal impairment may later contribute to raise calcium phosphate product metabolism.[1] The occurrence of visceral calcification in pseudohypoparathyroidism is a complicated issue. However, it has been postulated that visceral calcification exists as amorphous calcium phosphate.[2] It has been documented that serum phosphate >8-9 mg/dL and/or calcium phosphate product >70 mg/dL, local tissue injury, increased parathormone level—all contribute to heterotopic calcification.[3]

Nephrocalcinosis refers to bilateral minute calcifications in the renal pyramids due to calcium deposition in the tubular epithelium. It may occur in the absence of stones and is often diagnosed incidentally as in our asymptomatic patient with unremitting hypertension. Basal ganglia calcification as seen in our patient may occur physiologically in 0.3% to 1.5% of the population. It may also be found in hyper- and pseudohypoparathyroidism.[4]

The presence of calcinosis cannot be used as an index of neurological impairment, but its extent and the presence of decreased perfusion demonstrated by PET may be useful in predicting symptomatic progression.[5] Both hyper- and hypocalcemic states may give rise to metastatic calcifications. Incidental diagnosis of such calcifications should direct investigations towards parathyroid disorders.

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what's your diagnosis?

Rapidly growing nodule on the finger of a pregnant woman

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A 27-year-old woman, 28-weeks pregnant, presented with a rapidly growing nodule on her finger with spontaneous bleeding over the previous 4 weeks. Physical exam revealed a pedunculated vascular tumor on the left index finger (**Figure 1**). The most likely diagnosis is:

- Malignant melanoma
- Pyogenic granuloma
- Basal cell carcinoma
- Orf

FOR THE ANSWER, VISIT:
http://www.saudiannals.net/text.asp?2010/30/6/499/72283

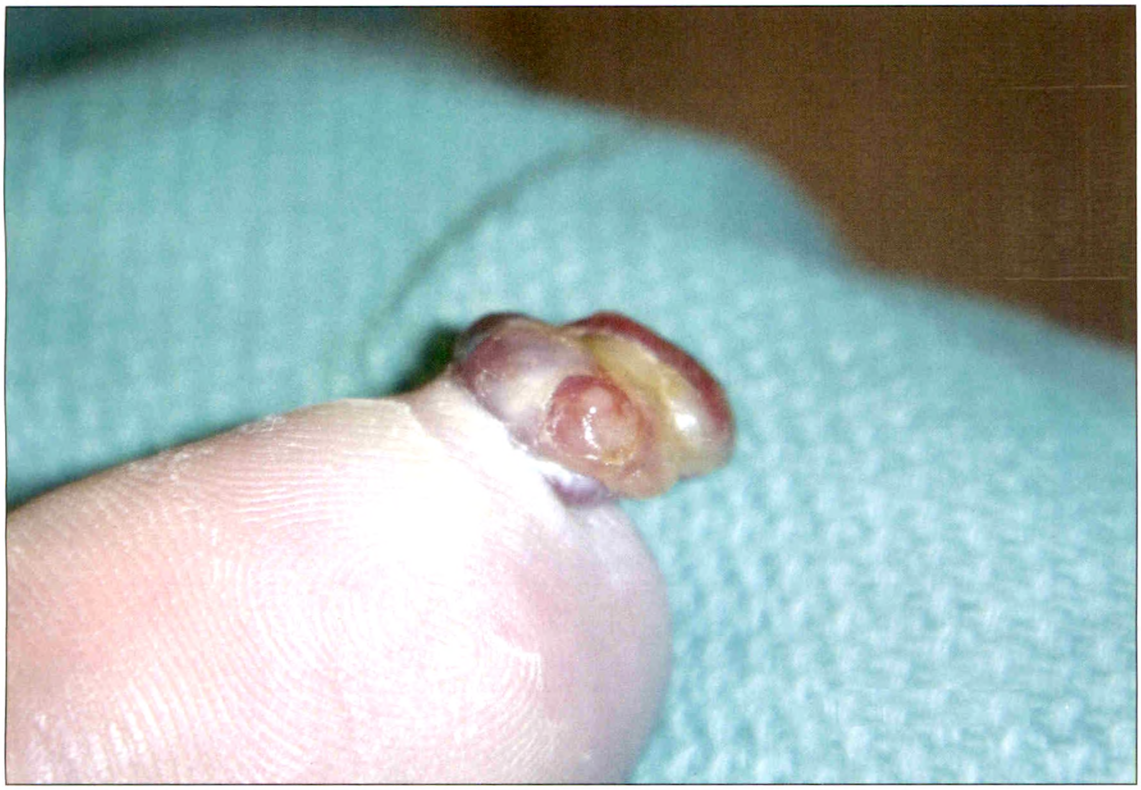

Figure 1. The nodule.

errata

El Mouzan MI, Foster PJ, Al Herbish AS, Al Salloum AA, Al Omer AA, Qurachi MM, Kecojevic T. Prevalence of overweight and obesity in Saudi children and adolescents. Ann Saudi Med 2010;30(3):203-208. PMID: 20427936. Some data in some of the tables (and the corresponding data in the text) are corrected as indicated. Changes are highlighted.

Table 1. Prevalence of overweight and obesity.

Age (years)	BMI > +1 SDS Number of children (%)			BMI > +2 SDS Number of children (%)			BMI > +3 SDS Number of children (%)		
	Boys	Girls	All	Boys	Girls	All	Boys	Girls	All
5–12	6149 (19.9)	5917 (19.2)	12066 (19.6)	6149 (9)	5917 (6.8)	12 066 (7.9)	6149 (2)	5917 (1)	12 066 (1.5)
13–18	3659 (24.8)	3592 (28.4)	7251 (26.6)	3659 (11.2)	3592 (10.0)	7251 (10.6)	3659 (2.6)	3592 (2.1)	7251 (2.4)
Overall	**9808** (21.7)	**9509** (22.7)	**19 317** (22.2)	**9808** (9.7)	**9509** (8.0)	**19 317** (8.9)	**9808 (2.3)**	**9509 (1.6)**	**19 317 (2.0)**

SDS: standard deviation scores

Table 4. Comparative prevalence of overweight using the WHO and CDC references.

Age (years)	2007 WHO Number of children (%)			2000 CDC Number of children (%)		
	Boys	Girls	All	Boys	Girls	All
5–12	6149 (19.9)	5917 (19.2)	12 066 (19.6)	6260 (15.9)	5985 (15.7)	12 245 (15.8)
13–18	3659 (24.8)	3592 (28.4)	7251 (26.6)	3253 (24.7)	3127 (25.1)	6380 (24.9)
Total	**9808** (21.7)[a]	**9509** (22.7)[a]	**19 317** (22.2)[b]	9513 (18.2)[a]	9112 (18.9)[a]	18 625 (18.6)[b]

[a] No statistically significant difference between boys and girls, bStatistically significant higher prevalence of overweight using the WHO reference ($P<.0001$) (The 2000 CDC reference underestimates the prevalence of overweight).

Table 5. Comparative prevalence of obesity using the WHO and CDC references.

Age (years)	2007 WHO Number of children (%)			2000 CDC Number of children (%)		
	Boys	Girls	All	Boys	Girls	All
5–12	6149 (9)	5917 (6.8)	12066 (7.9)	6260 (5)	5985 (3.9)	12 245 (4.5)
13–18	3659 (11.2)	3592 (10)	7251 (10.6)	3253 (8.2)	3127 (5.5)	6380 (7)
Total	**9808** (9.7)[a]	**9509** (8.0)[a]	**19 317** (8.9)[b]	**9513** (6.0)[a]	**9112 (4.7)**[a]	**18 625** (5.4)[b]

[a] No statistically significant difference between boys and girls, [b] Statistically significant higher prevalence of overweight using the WHO reference ($P<.0001$) (The 2000 CDC reference underestimates the prevalence of obesity).

El Mouzan et al. Prevalence of malnutrition in Saudi children: a community-based study. Ann Saudi Med 2010;30(5):381-385. PMID: 20697172. Some data in some of the tables (and the corresponding data in the text) are corrected as indicated. Changes are highlighted.

Table 1. Prevalence of underweight by age and gender.

Age (years)	Total number (%) <–2 SD			Total number (%) <–3 SD		
	Boys	Girls	Combined	Boys	Girls	Combined
<1	4343 (5.1)	4141 (2.3)	8484 (3.7)	4343 (1.1)	4141 (0.5)	8484 (0.8)
1 to < 2	1283 (5.5)	1228 (2.9)	2511(4.2)	1283 (0.8)	1228 (0.4)	2511 (0.6)
2 to < 3	703 (7.8)	727 (7.2)	1430 (7.5)	703 (1)	727 (1.5)	1430 (1.3)
3 to < 4	716 (7.8)	787 (8.8)	1503 (8.3)	716 (2)	787 (1.5)	1503 (1.8)
4 to < 5	845 (10.3)	828 (11.1)	1677 (10.7)	845 (2.1)	828 (1.6)	1673 (1.9)
Overall	**7890** (6.1)[a]	**7711** (4.5)[a]	**15601** (5.3)	**7890** (1.2)[b]	**7711** (0.8)[b]	**15601** (1.0)

[a] *P*<.0001, [b] *P*=.0154 [a] Significant difference in prevalence of moderate underweight between boys and girls (*P*<.0001). [b] Significant difference in prevalence of severe underweight between boys and girls (*P*=.0154).

Table 2. Prevalence of wasting by age and gender.

Age (years)	Total number (%) <–2 SD			Total number (%) <–3 SD		
	Boys	Girls	Combined	Boys	Girls	Combined
<1	4290 (12.3)	4109 (10.8)	8399 (11.6)	4290 (5.6)	4109 (4.4)	8399 (5)
1 to < 2	1282 (6.9)	1227 (4.5)	2509 (5.7)	1282 (1.9)	1227 (1.6)	2509 (1.8)
2 to < 3	698 (12.3)	727 (8.4)	1425 (10.4)	698 (3.2)	727 (2.8)	1425 (3)
3 to < 4	714 (10.5)	785 (9)	1499 (9.8)	714 (2.5)	785 (1.9)	1499 (2.2)
4 to < 5	893 (12.6)	826 (10.8)	1719 (11.7)	893 (3.5)	826 (1.9)	1719 (2.7)
Overall	7823 (12.7)[a]	**7674** (10.8)[a]	15497 (11.8)	7823 (5.1)[a]	**7674** (3.9)[a]	15497 (4.5)

[a] *P*<.0001

Table 3. Prevalence of stunting by age and gender.

Age (years)	Total number (%) <–2 SD			Total number (%) <– 3 SD		
	Boys	Girls	Combined	Boys	Girls	Combined
<1	4336 (10.5)	4123 (5.2)	8459 (7.9)	4336 (3.3)	4123 (1.5)	8459 (2.4)
1 to < 2	1278 (12.5)	1225 (11.1)	2503 (11.8)	1278 (3.8)	1225 (3.3)	2503 (3.6)
2 to < 3	703 (14.1)	726 (10.1)	1429 (12.1)	703 (3.4)	726 (3.4)	1429 (3.4)
3 to < 4	716 (11.7)	787 (12.8)	1503 (12.3)	716 (3.4)	787 (2.5)	1503 (3.0)
4 to < 5	845 (10.1)	829 (10.3)	1674 (10.2)	845 (1.8)	829 (1.9)	1674 (1.9)
Overall	**7878** (10.8)[a]	**7690** (7.8)[a]	**15568** (9.3)	**7878 (3.1)**[a]	**7690** (2.1)[a]	**15568** (2.6)

[a] *P*<.0001

Table 4. Comparative national prevalence of nutritional indicators in selected countries.

Country	Study years	Sample size	Underweight (%)		Wasting (%)		Stunting (%)	
			<–2 SD	<–3 SD	<–2 SD	<–3 SD	<–2 SD	<–3 SD
Bangladesh[19]	2006	24 302	39.8	10.3	12.4	1.6	47	15.5
Yemen[20]	2003	12 364	43.1	18.5	15.2	6.3	57.7	35.8
Brazil[21]	2006-07	4415	2.2	0.3	4.0	1.6	7.1	1.5
USA[17]	1999-04	3920	1.3	0.2	0.6	0.1	3.9	0.5
Egypt[22]	2005	12 830	5.4	1.7	5.3	2.5	23.8	10.3
Nigeria[23]	2003	5407	27.9	11.9	11.2	4.8	43	23.7
Indonesia[24]	2007	77 808	19.6	5.1	14.8	6.8	40.1	21
Oman[25]	1999	14 076	11.3	1.6	7.3	1.1	12.9	2.4
Present study	2004-05	15 601	5.3	1.0	11.8	4.5	9.3	2.6

Somily AM. An imported enteric fever caused by a quinolone-resistant *Salmonella typhi*. Ann Saudi Med [serial online] 2010 [cited 2010 Sep 5];30:313-6. Available from: http://www.saudiannals.net/text.asp?2010/30/4/313/65267

Incorrect *Salmonella* terminology was applied. The term *Salmonella* Typhi (and abbreviated form *S* Typhi) should have been used in the title and elsewhere, according to current nomenclature (http://www.bacterio.cict.fr/salmonellanom.html; AMA Manual of Style, 10th edition, Section 15.4.2).

King Faisal Specialist Hospital & Research Centre

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World Health Organization
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Directory of Health, Medical Conferences and Professional Meetings in the Region

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Objectives

To develop and maintain a directory of conferences and meetings organized by government institutions, academic bodies and other non-profit professional organizations in the Region.

The directory will:

- Serve as a vehicle to distribute information on these events to health care professionals and academicians;
- Enhance networking and collaboration between institutions;
- Record professional events for the future reference and archiving of conference proceedings as much as possible;
- Alert health care professionals and academicians to the new conferences related to their speciality.

Section of Colorectal Surgery Fellowship Training Program

A two-year clinical fellowship program that aims to provide the candidate with the basic science, clinical and operative skills to manage patients with colorectal diseases competently. The operative load of the Section is 300 operations annually. The majority of such operations are complex colorectal conditions such as colorectal cancer, fecal incontinence, complex anal fistulae and complicated inflammatory bowel disease conditions. The fellowship provides training into diagnostic and therapeutic colonoscopy such as insertion of colonic stents for bowel obstruction. Moreover, training into endoanal and endorectal ultrasound for the assessment of anal sphincters and staging of rectal cancer will be provided, respectively. An important aspect of the program is training into anorectal physiology testing and the interpretation of such complex data. During training, the fellow will be exposed to Enterostomal therapy and all aspects of stoma care. The Section holds an annual colorectal surgery symposium and the fellow is expected to be an active member in this activity, which includes presentation and publication of medical literature in the area of colorectal surgery. Laparoscopic colorectal surgery will be an integral part of training along with standard open surgery. The Section has a well-established curriculum of teaching activities such as morbidity and mortality meetings, radiology rounds and GI clubs. The fellow is expected to participate actively in these educational activities.

For interested applicants, please
contact:Academic & Training Affairs
T: 442-7214/5
ata@kfshrc.edu.sa

The Palliative Medicine Fellowship Program

King Faisal Specialist Hospital & Research Centre, Riyadh
One-Year Fellowship Program

Applicants:

1. Must be Saudi nationals.
2. Must be Saudi Board (or equivalent) certified in one of the following specialties:

Family Medicine, Internal Medicine, Anesthesiology, General Surgery
Boards in other specialites may be considered on indiviudal basis.

For information, please contact: Academic & Training Affairs; King Faisal Specialist Hospital & Research Centre; P.O. Box 3354, MBC 36; Riyadh 11211, Kingdom of Saudi Arabia. T: +966-1-464 7272 ext. 31833; F: +966-1-442 7237

announcements

Hepatobiliary-Pancreatic Surgery Fellowship Program

KFSH&RC-Riyadh, Saudi Arabia

Hepatobiliary-Pancreatic Surgery Fellowship Program is a compherehensive structured educational program designed to graduate independent surgeons with high standards of skills, broad knowledge and proficient in clinical decisions. The fellow will attain proficiency in endoscopic procedures, be able to interpret radiological investigations exam as well a take care of basic and complex hepatobiliary-pancreatic surgical procedures.

The Department of Liver Transplantation and Hepatobiliary-Pancreatic Surgery at King Faisal Specialist Hospital & Research Center offers:

- Four qualified hepatobiliary and transplant consultant surgeons with good experience in teaching and commitment to provide academic teaching and training.
- A very good facility in interventinal radiology.
- Equipped Operating rooms and intensive care facility.
- Outstanding internet access for library service for research resources as well as outstanding research facility and database available for research users.

The Fellowship Program is for two years and there are two positions available for application. Qualified candidates can direct their query to:

Academic & Training Affairs
KFSH&RC-Riyadh (MBC 36)
P.O. Box 3354, Riyadh 11211, Saudi Arabia
T: +966-1-442-7215/442-7214
F: +966-1-442-7237
ata@kfshrc.edu.sa

links for authors and reviewers

Good reporting of trials and observational studies: Related guidelines and initiatives

EQUATOR

EQUATOR <http://www.equator-network.org> (Enhancing the QUAlity of Trials and Other Research) The EQUATOR Network is a new initiative that seeks to improve the quality of scientific publications by promoting transparent and accurate reporting of health research.

STROBE

STROBE **(STrengthening the Reporting of OBservational studies in Epidemiology)** is an international, collaborative initiative of epidemiologists, methodologists, statisticians, researchers and editors involved in the conduct and dissemination of observational studies.

CONSORT (Consolidated Standards of Reporting Trials), is part of a broader push to produce reporting guidelines (RGs) for many different types of research. The main product of CONSORT is the CONSORT Statement, which is an evidence-based, minimum set of recommendations for reporting RCTs. It offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation. The CONSORT Statement comprises a 22-item checklist and a flow diagram, along with some brief descriptive text.

ASSERT

The ASSERT **(A Standard for the Scientific and Ethical Review of Trials)** initiative is a proposed standard for the review and monitoring of randomized clinical trials by research ethics committees. Among other items, its checklist incorporates certain elements of CONSORT, to ensure fulfillment of the requirements for scientific validity in the ethical conduct of clinical research.

MOOSE

The MOOSE **(Meta-analysis of Observational Studies in Epidemiology)** paper reports the outcome of a 1997 workshop to examine the reporting of meta-analyses of observational studies. It makes recommendations to aid authors, editors, reviewers and readers of observational research.

QUOROM

The QUOROM **(Quality Of Reporting Of Meta-analyses)** conference resulted in the creation of the QUOROM Statement, which consists of a checklist and flow diagram.

STARD

The STARD **(Standards for Reporting of Diagnostic Accuracy)** initiative aims to improve the accuracy and completeness of reporting of studies of diagnostic accuracy in order to allow readers to assess the potential for bias in a study and to evaluate the generalisability of its results.

تأثير رأب اللهاة والحنك والبلعوم التقليدي وبالليزر على الصوت واللفظ الأنفي

مقدمة: يُعتبر رأب اللهاة والحنك والبلعوم من الإجراءات الجراحية الشائعة لإعادة ترميم الفم والحنك عند مرضى انقطاع النفس ألانسدادي أثناء النوم. ويمكن أن يتم ذلك من خلال الرأب التقليدي أو بالليزر. وقد سعت هذه الدراسة إلى تقييم تأثير كل من الرأب التقليدي والرأب بالليزر على جودة الصوت ووضوح الكلام.

المرضى والطرق: اشتملت الدراسة على ٢٧ مريضاً، بلغ متوسط أعمارهم ٤٦ عاماً. وشُخصت حالاتهم بأنها انقطاع النفس ألانسدادي أثناء النوم بموجب فحص تخطيط النوم. وقد وزع المرضى على مجموعتين طبقاً لنوعية الإجراء الجراحي. خضع ١٥ مريضاً للرأب التقليدي و١٢ مريضاً للرأب بالليزر. وجرى تقييم شامل لجميع المرضى قبل وبعد الجراحة تضمن التقييم الإدراكي السمعي للصوت والكلام، والتقييم الموضوعي باستخدام التحليل الصوتي وقياس اللفظ الأنفي.

النتائج: لم يُظهر كل من التقييم الإدراكي السمعي للكلام والصوت، والتحليل السمعي للصوت. وتحليل اللفظ الأنفي للكلام أي فروق إحصائية هامة بين التقييمات السابقة والتالية للجراحة في كلتا المجموعتين (P› ٠٥).

الاستنتاج: أظهرت نتائج هذه الدراسة عدم وجود تأثيرات هامة على جودة الصوت أو حصيلة الكلام عند مرضى انقطاع النفس ألانسدادي أثناء النوم سواء من خضع منهم لرأب اللهاة والحنك والبعلوم التقليدي أو بالليزر.

التغيرات السكرية بعد تعزيز فيتامين د عند مرضى النوع الأول من داء السكري وعوز فيتامين د

مقدمة: أُجريت تجربة استطلاعية ضابطة غير عشوائية وغير معماة لفحص الفرضية القائلة بأن تعزيز الفيتامين د قد يُحسن من السيطرة على السكر عند مرضى النوع الأول من داء السكري الذين يعانون من عوز الفيتامين د.

المرضى والطرق: تم تحديد ثمانين مريضاً من المصابين بالنوع الأول من داء السكري ممن كانت مستويات ٢٥-هدركسي فيتامين د لديهم أقل من ٥٠ L/nmol لتلقي ٤٠٠٠ وحدة دولية من فيتامين د٣. وأُضيفت مكملات الكالسيوم لضمان مدخول إجمالي من الكالسيوم بمقدار ١٢٠٠ mg/d. وجرى قياس مستويات غليوكوزيد الهيموغلوبين و٢٥-هدركسي فيتامين د منذ خط الأساس وبعد ١٢ أسبوعاً.

النتائج: ظهر هناك فرق هام في متوسط (الانحراف المعياري) لمستوى غليوكوزيد الهيموغلوبين (٪) بين المجموعات التي حققت مستويات ٢٥-هدركسي فيتامين د بلغت ٣٥,٤، L/nmol ٣٥,٤-٥١ L/nmol و٥١، L/nmolبعد ١٢ أسبوعاً (p=٠٢). كان هناك فرق هام في تغير غليوكوزيد الهيموغلوبين من الخط الأساس بين المجموعات التي حققت ٢٥-هدركسي فيتامين د بلغت ٣٥,٤، L/nmol. ٣٥,٤-٥١ L/nmol و٥١، L/nmolبعد ١٢ أسبوعاً (p=٠٤). كان هناك فرق واضح في مستوى ٢٥-هدركسي فيتامين د بين المجموعات التي حققت مستويات غليوكوزيد الهيموغلوبين ٧,٨، ٧,٨-٩,٩ و٩,٩، بعد ١٢ أسبوعاً (P=٠٠١). وكان من المرجح أن يحقق المرضى مستويات أدنى من غليوكوزيد الهيموغلوبين بعد ١٢ أسبوعاً إذا كان لديهم مستويات أعلى من ٢٥-هدركسي فيتامين د بعد ١٢ أسبوعاً (r=٠,٤، P=٠٠١).

الاستنتاج: ظهر تأثير ملحوظ لتعزيز فيتامين د على ضبط السكر عند مرضى النوع الأول من داء السكري الذين يعانون من عوز الفيتامين د. هناك حاجة لمزيد من الدراسات لمعرفة إمكانية تطبيق هذه المشاهدات.

نوعية الأمراض الجلدية في منطقة القصيم بالمملكة العربية السعودية: ما الذي يجب على طبيب الرعاية الأولية معرفته

مقدمة: إن الدراسات الوبائية التي توضح حجم الأمراض الجلدية ضرورة للتخطيط السليم للرعاية الصحية. كان الهدف من هذه الدراسة هو البحث عن نوعية الأمراض الجلدية لدى مرضانا من المراجعين لعيادات الأمراض الجلدية التابعة للجامعة في منطقة القصيم.

المرضى والطرق: أجرينا دراسة استباقية على جميع المرضى السعوديين من مراجعي عيادات وزارة الصحة للأمراض الجلدية التابعة لكلية الطب بجامعة القصيم لمدة ١٢ شهراً منذ ١ مارس ٢٠٠٨م وحتى ٢٨ فبراير ٢٠٠٩م.

النتائج: شملت الدراسة ٣٠٥١ مريضاً بلغ عدد الذكور منهم ١٧٨٦ (٥,٥٨٪) والإناث ١٢٦٥ (٥,٤١٪). أي أن عدد الذكور فاق عدد الإناث (P < ٠,٠٥) (معدل الذكور إلى الإناث، ١,٤:١). وكان متوسط أعمار المرضى (الخطأ المعياري للمتوسط) ٣,٢٥ (٢٧,٠). وبلغت نسبة المرضى الذين تتراوح أعمارهم بين ٥ و٣٤ عاماً ٧١٪ تقريباً. أما أكثر خمسة أمراض جلدية شيوعاً فهي: الأكزيما/ التهاب الجلد (٥,١٩٪)، والالتهابات الفيروسية (٦,١٦٪)، والاضطرابات الشعرية الدهنية (٤,١٤٪)، والآفات التصبغية (٢,١١٪)، واضطرابات الشعر (٦,٧٪). وأكثر الاضطرابات شيوعاً لدى الذكور هي التهابات الجلد الفيروسية (٠,٢٠٪)، بينما كانت الأكزيما/ التهاب الجلد (٧,٢٠٪) أكثر أمراض الجلد انتشاراً بين الإناث. سجلنا تغيرات موسمية في حالات الآفات التصبغية والاضطرابات الحطاطية الحرشفية والالتهابات بالأوالي.

الاستنتاج: تُعد الأمراض الجلدية المعدية، والأكزيما/ التهاب الجلد، والاضطرابات الشعرية الدهنية، والآفات التصبغية، واضطرابات الشعر أكثر خمسة أمراض جلدية شيوعاً. ويجب المبادرة بعمل برامج تدريب مناسبة لأطباء الرعاية الأولية والممارسين العامين الآخرين لتشخيص وعلاج الأمراض الجلدية الشائعة وذلك لتقليل الإحالات إلى عيادات الأمراض الجلدية.

مقارنة بين مستويات المصل الأساسي ومستويات السائل الجريبي للهرمون مضاد المولري كمنبئ لنتائج الإخصاب في الأنبوب لدى المريضات الآتي يعانين من متلازمة المبيض متعدد الأكياس والمريضات الآتي لا يعانين منها

مقدمة: ينال التنبؤ بنتائج الإخصاب في الأنبوب (IVF) عن طريق قياس الهرمون مضاد المولري (AMH) اهتماماً متزايداً من قبل الاختصاصيين السريريين. وتقارن هذه الدراسة العلاقة بين مستويات الهرمون مضاد المولري في المصل أو داخل الجريب ونتائج الإخصاب في الأنبوب لدى النساء اللآتي يعانين من متلازمة المبيض متعدد الأكياس (PCOS) والنساء الآتي لا يعانين منها.

المرضى والطرق: أجريت هذه الدراسة الاستباقية في عيادتَي إخصاب تابعتين لمستشفيات جامعية. وتم جمع عينات المصل في اليوم الثالث من الدورة أما السائل الجريبي (FF) فتم جمعه يوم استخراج البويضة من ٢٦ سيدة يعانين من متلازمة المبيض متعدد الأكياس و٤٢ سيدة إباضتهن سليمة يمثلن المجموعة الضابطة. تم قياس مستويات الهرمون مضاد المولري في العينات باستخدام المقايسة المناعية الإنزيمية. وأجرينا مزيداً من التقييم للعلاقة بين مستويات الهرمون مضاد المولري في المصل أو السائل الجريبي ونتائج الإخصاب في الأنبوب، بما في ذلك عدد البويضات المستخرجة، ومعدل نضج البويضات، ومعدل الإخصاب، ومعدل الإنغراس، ومعدل الأجنة ذات درجة الجودة العالية، ومعدلات الحمل السريرية والكيمياحيوية.

النتائج: كان متوسط مستويات المصل الأساسي والسائل الجريبي للهرمون مضاد المولري أعلى بكثير في مجموعة متلازمة المبيض متعدد الأكياس مقارنة بالمجموعة الضابطة، وكانت القيم على التوالي هي ٢,١٤ نانوغرام/مل مقابل ٢,٣ نانوغرام/مل (P < ٠٠١,٠) و٢,٨ نانوغرام/غم بروتين مقابل ٧,٤ نانوغرام/غم بروتين (P < ٠١,٠). وظهرت في كلا المجموعتين علاقة متبادلة إيجابية بين مستويات المصل الأساسي للهرمون مضاد المولري وعدد البويضات المستخرجة (r= ٣٢٣,٠؛ P= ٠٣٧,٠ في المجموعة الضابطة مقابل r= ٥٢٩,٠؛ P= ٠٠٥,٠ في مجموعة متلازمة المبيض متعدد). وكانت هناك علاقة إيجابية في المجموعة الضابطة بين مستويات المصل الأساسي للهرمون مضاد المولري ونسبة البويضات الناضجة (r= ٣٣١,٠؛ P= ٠٣٢,٠) ومعدل الإنغراس (r= ٣٠٥,٠؛ P= ٠٥,٠).

الاستنتاج: يمكن أن تكون مستويات المصل الأساسي للهرمون مضاد المولري، وليس داخل الجريب، عاملاً منبئاً جيداً للنتائج الكمية والنوعية للإخصاب في الأنبوب لدى السيدات اللآتي يتمتعن بإباضة سليمة، ولكن ليس لدى النساء اللآتي يعانين من متلازمة المبيض متعدد الأكياس.

ترابط تعدد أشكال بيتا إنترلوكين-١ (-٥١١C/T) مع تخلخل العظم في النساء بعد انقطاع الطمث

مقدمة: يعتبر تخلخل العظم داء شائعاً في كبار السن حيث تساهم العوامل الجينية والسريرية في النمط الظاهري للداء. وحيث أن إنتاج الإنترلوكين-١ مشارك في اضطرابات كتلة العظم والهيكل. فقد قمنا باجراء استقصاء ما إذا كان تعدد أشكال جين نظام الإنترلوكين-١ مرتبطاً بأمراض تخلخل العظم في نساء تايوان بعد انقطاع الطمث.

المرضى والطرق: يتم تشخيص تخلخل العظم عن طريق قياس الامتصاص بالأشعة السينية مزدوجة الطاقة. والتي تقوم بقياس الكثافة المعدنية للعظم في عدة مواقع للهيكل العظمي. قمنا بدراسة إنترلوكين-١أ (-٨٨٩C/T). و إنترلوكين-١ب (-٥١١C/T) بالإضافة إلى (VNTR) لقاعدة الزوج ٨٦ في الإنترون ٢ لجين ضادة مستقبل إنترلوكين- ١ (IL-ra١) في ١١٧ امرأة مصابات بتخلخل العظم بعد انقطاع الطمث و١٣٥ من مرضى المجموعة الضابطة لا يعانون من أعراض تخلخل العظم. تم تحليل تعدد أشكال الجين بواسطة طريقة (PCR-RFLP). كما تم تحديد السكر وعوامل الخطر الأخرى.

النتائج: أظهر تكرار الأنماط الجينية اختلافاً إحصائياً واضحاً بين المجموعتين للإنترلوكين-١ب (P-) (٥١١C/T)=٠.٠٢٢ .النسبة الأرجحية=١.٩٧٢) والألائل (P=٠.٠٢ . النسبة الأرجحية=٢.٩٠٩). ولكننا لم نعثر على أي اختلاف إحصائي واضح في تعدد أشكال الإنترلوكين-١أ و الإنترلوكين-١ب (P،٠.٠٥). كما لاحظنا علاقة ايجابية بين تخلخل العظم والكوليسترول وعلاقة عكسية ضعيفة بين سكر الدم وتخلخل العظم في النساء بعد انقطاع الطمث.

الاستنتاج: توحي هذه النتائج التجريبية بترابط أمراض تخلخل العظم مع تعدد أشكال الإنترلوكين-١ب (-٥١١C/T) في النساء بعد انقطاع الطمث. يعتبر تعدد الأشكال عامل خطر مستقل لتخلخل العظم.

تعدد أشكال جين K٤٦٩E لجزيء التصاق الخلايا-١ في المصريين الذين يعانون من داء قلبي إكليلي

مقدمة: إن الخطوة الأولى في التصلب العصيدي هي التصاق الكريات البيضاء بخلايا بطانية نشطة بواسطة جزيء التصاق الخلايا -١ (ICAM-١). تهدف هذه الدراسة استقصاء ارتباط تعدد أشكال جين التصاق الخلايا-١ K٤٦٩E ومستوى ICAM-١ (sICAM-١) الجزيء الذواب في المصل مع داء القلب الإكليلي في المرضى المصريين.

المرضى والطرق: باستخدام تصميم الحالات والشواهد . قمنا باجراء دراسة على ١٠٠ مريض يعانون من داء قلبي إكليلي، منهم ٣٧ مريضاً يعانون من احتشاء عضلي قلبي حاد (MI) و٢٧ مريضاً يعانون من ذبحة صدرية غير مستقرة (UA). وقد تكونت المجموعة الشاهدة على ٥٠ شخصاً بحالة صحية جيدة لديهم وظيفة طبيعية للبطين الأيسر. صنف النمط الجيني لجميع المشاركين بالنسبة لتعدد أشكال جزيء التصاق الخلايا -١ بواسطة طريقة (PCR-RFLP). تم قياس الجزيء الذواب sICAM-١ في المصل بواسطة مقايسة مناعية مرتبطة بالإنزيم (ELISA).

النتائج: كان تكرار النمط الجيني K (KK,EK) في المرضى الذين يعانون من الداء القلبي الإكليلي أعلى بشكل واضح عند مقارنتها بالمجموعة الشاهدة (P<.۰۰۱) ومترابط مع تزايد الخطورة للإصابة بالداء (OR=۳,۸,۹۵٪ CI: ۱,۷ to ۸,۵; P=.۰۰۱). لم يظهر تكرار النمط الجيني K في المرضى الذين يعانون من احتشاء عضلي قلبي أي اختلافاً كبيراً عند مقارنته بالمرضى الذين يعانون من ذبحة صدرية غير مستقرة (P=.۱۲۱). كانت مستويات sICAM-١ في المصل قابلة للمقارنة بين المرضى الذين يعانون من الداء القلبي الإكليلي والمجموعة الضابطة (P=.۳۷) وبين المرضى الذين يعانون من احتشاء عضلي قلبي و ذبحة صدرية غير مستقرة (P=.۲۳). لا توجد أي اختلافات واضحة في مستويات sICAM-١ في المصل لدى المرضى الذين يحملون أنماطاً جينية مختلفة (P=.۵۳۲). إن مستويات sICAM-١في المصل أعلى في الرجال من النساء (P=.۰۰٤).

الاستنتاج: إن تعدد أشكال جين جزيء التصاق الخلايا-١ في الرامزة ٤٦٩ مرتبطة بخطر إصابة المصريين بداء القلب الإكليلي. كما أن الجزيء الذواب sICAM-١ في المصل لا يتأثر بتعدد الشكل هذا وليس بالضرورة مرتفعاً في الداء القلبي الإكليلي.

المدونـــات الطبيـــة السعوديـــة

تصدر المدونات الطبية السعودية مرة كل شهرين عن مستشفى الملك فيصل التخصصي ومركز الأبحاث . ص.ب ٣٣٥٤ . الرياض ١١٢١١. المملكة العربية السعودية. يتم توزيع المدونات وإعداد الملخصات العربية بالتعاون مع د. زهير القاوي بواسطة موظفي إدارة الخدمات الإدارية بمستشفى الملك فيصل التخصّصي ومركز الأبحاث. جميع المراسلات والاستفسارات وطلبات الاشتراك ترسل إلى المحرر الأول مكتب المطبوعات (ر.إ ٣٦).

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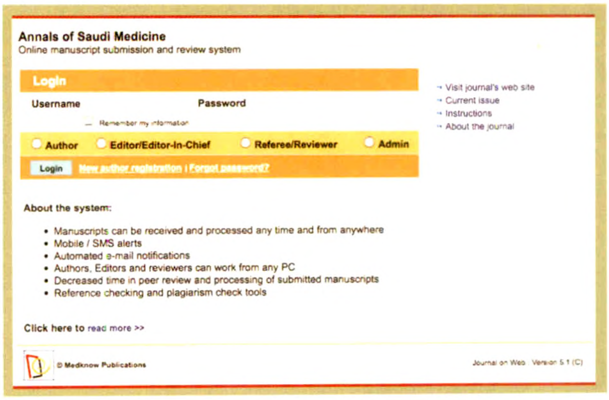
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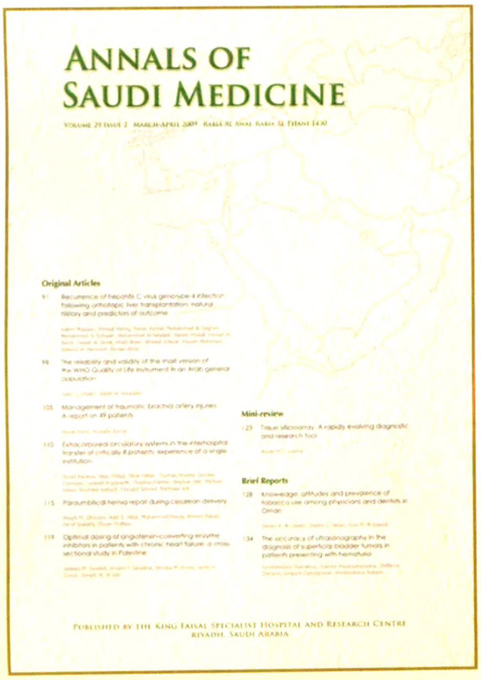
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